In Utero and Early Life Susceptibility to Carcinogens:

The Derivation of Age-at-Exposure Sensitivity Measures

June 2008

California Environmental Protection Agency
Office of Environmental Health Hazard Assessment
Reproductive and Cancer Hazard Assessment Branch

STUDY AUTHORS

Martha S. Sandy, Ph.D., M.P.H., Chief
Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch

Claire D. Sherman, Ph.D.
Biostatistician
Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch

Rajpal S. Tomar, Ph.D.
Staff Toxicologist
Cancer Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch

Lauren Zeise, Ph.D., Chief
Reproductive and Cancer Hazard Assessment Branch

STUDY REVIEWERS

John Budroe, Ph.D.
Staff Toxicologist
Air Toxicology and Risk Assessment Section
Air Toxicology and Epidemiology Branch

Andy Salmon, M.A., D. Phil., Chief
Air Toxicology and Risk Assessment Section
Air Toxicology and Epidemiology Branch

Melanie Marty, Ph.D., Chief
Air Toxicology and Epidemiology Branch

Robert Howd, Ph.D., Chief
Water Toxicology Section
Pesticide and Environmental Toxicology Branch

George V. Alexeeff, Ph.D.
Deputy Director for Scientific Affairs

Allan Hirsch
Chief Deputy Director

The contributions of Daniel Sultana, M.S. and Lindsey Roth, M.A. in preparing many of this report's figures, and the contributions of Thomas McDonald, Ph.D., Kate MacGregor, M.P.H., Safie Yaghoubi, M.S., and Robert Schlag, M.S. to the identification and initial evaluation and analysis of animal cancer studies with early life exposure to carcinogens are gratefully acknowledged.

TABLE OF CONTENTS

Executive Summary

Early-in-life susceptibility to carcinogens has long been recognized by the scientific community and clinicians as a public health concern. Numerous scientific publications and symposia have addressed this issue over the years and the scientific literature contains a number of human clinical findings and epidemiological studies of early life cancer susceptibility. While there are many indications of increased human cancer susceptibility in early life, the magnitude of the impact has been difficult to gauge, and until recently risk assessment procedures have not in general addressed the issue. The California legislature in 2000 recognized the need for a systematic approach to develop scientifically based methods to address this concern so that in environmental decision making special sensitivities of the developing fetus, and the young were taken into account. The legislature directed the Office of Environmental Health Hazard Assessment (OEHHA) to assess methodologies used in addressing early-in-life risk, compile animal data to evaluate those methods, and develop methods to adequately address carcinogenic exposures to the fetus, infants, and children (Children's Environmental Health Initiative [AB 2872, Shelly]; California Health and Safety Code [HSC] section 901 [a] through [e]).

In 2001, OEHHA assessed cancer risk assessment methodologies, and concluded that the existing risk assessment approaches did not adequately address the possibility that risk from early-in-life exposures may differ from that associated with exposures occurring in adulthood. OEHHA further concluded that there was a need for methodologies addressing early-in-life cancer risk to be developed, tested, and validated.

Also in 2001, OEHHA began compiling animal cancer studies with early life exposure to carcinogens. Two types of studies with early life exposure were compiled. The first type, "multi-exposure window studies," have exposure groups in at least one of the following age groups − prenatal (from conception to birth), postnatal (from birth to weaning), juvenile (from weaning to sexual maturity) − along with an older age-at-exposure reference group. The second type supports the "case study" of individual chemicals. It includes experiments with at least one group of animals dosed solely during one of the three early age-at-exposure windows named above. Case studies are constructed from multiple such experiments in each of these early life exposure windows.

This document presents 1) the statistical methods developed and used to systematically analyze the data from multi-exposure window studies and case studies to derive measures of early-life susceptibility; 2) the results of applying these analyses to multi-exposure window studies on 23 unique carcinogens and two case studies on diethylnitrosamine (DEN) and ethylnitrosourea (ENU); and 3) conclusions regarding the sensitivity of the fetus, infants, and children to carcinogen exposures.

Analytical Approach

Analysis of the data involved the derivation of a cancer potency, that is, the slope of the dose response curve, for each of the experiments selected. When treatment related tumors were observed at multiple sites in an experiment, or at the same site, but arising from different cell types, slopes from these different sites or types were statistically combined to create an overall multisite cancer potency for that experiment. The ratio of cancer potency derived from an early life exposure experiment to that derived from an experiment conducted in adult animals, referred to here as an age sensitivity factor (ASF), was taken as a measure of early-life susceptibility. Two types of ASFs are developed for each early life age window: An unadjusted and an adjusted ASF. The unadjusted ASF focuses on the inherent susceptibility of the young to the carcinogen and considers potencies for individuals followed for similar periods of time and similarly exposed but for the age window in which the exposure occurs. Thus the unadjusted ASF does not address the longer period of time that carcinogen exposure to the young has to manifest as cancer, also referred to as the longer "shelf-life" (or expected years of life remaining) of the carcinogen-exposed fetus, infant, or child, as compared to the shorter "shelf-life" of the carcinogen-exposed adult. Application of a time-of-dosing adjustment based on the Doll-Armitage model of carcinogenesis is then applied to address this issue of "shelf-life." The resulting "adjusted ASF" addresses both the inherent susceptibility to the young to some carcinogens as well as the "shelf life" issue.

Prenatal, postnatal and juvenile ASFs were developed for the 23 carcinogens with multi-window experiments and similarly, ratios of potencies for "early life" to "later life" exposures (e.g., prenatal:juvenile and postnatal:juvenile) were developed for the case studies.

Characteristics of the Chemicals Studied

Twenty of the 23 carcinogens included in the multi-exposure window analyses are considered to act via primarily genotoxic modes of action, with 16 thought to require metabolic activation to the ultimate carcinogenic species. Fourteen carcinogens, including one thought to act via primarily nongenotoxic modes of action, were included in the multi-window studies with prenatal exposure groups; 18 carcinogens, including two thought to act via primarily nongenotoxic modes of action, were included in the multi-window studies with postnatal exposure groups; and five carcinogens were included in the multi-window studies with juvenile exposure groups. The case study chemicals, DEN and ENU, are both genotoxic. ENU is a direct acting alkylating agent, while DEN requires metabolic activation.

Results

The results of the multi-window and case study analyses indicate that the prenatal, postnatal, and juvenile lifestages can be much more susceptible to developing cancer than the adult lifestage. While there is a great deal of variability across the chemicals studied in the prenatal ASFs, the potency associated with prenatal carcinogen exposure is not zero. Median estimates of prenatal ASFs from the multi-window analysis, adjusted to take into account the longer period for cancer to manifest after early life exposures, range from 2.8 to 7.5 and mean estimates from 16.6 to 37.1, depending on the method used to combine studies. The DEN and ENU case studies illustrate the variability across chemicals in the sensitivity of the prenatal period, with results for DEN suggesting inherently less sensitivity than older animals from *in utero* exposure, and for ENU just the opposite. ENU does not require metabolic activation, whereas DEN does and cannot be metabolized to any significant extent by fetal tissues until relatively late in gestation. This may explain the lower fetal susceptibility of DEN. However, the multi-exposure window studies illustrate that *in utero* metabolic status is not the sole determinant of *in utero* susceptibility: benzidine and safrole require metabolic activation and exhibit greater susceptibility from *in utero* exposure.

In the case of exposures occurring during the postnatal age window, the data indicate an inherently greater susceptibility compared to the adult period. Median unadjusted ASFs are 4.6 – 7.4 and mean estimates are 27.1 – 42.4, depending on the method used to combine studies. The increased susceptibility appears even more pronounced once adjustments are made to take into

account the long period cancer has to manifest when exposure occurs early in life. Median estimates of postnatal adjusted ASFs from the multi-window analysis range from 13.4 to 21.6 and mean estimates from 78.5 to 123.1, depending on the method used to combine studies. The DEN and ENU case studies also exhibit substantial sensitivity during the postnatal period.

While there were just five chemicals and seven studies, two of which were not independent, available to examine susceptibility in the juvenile period, significantly greater susceptibility compared to the adult period was observed in three of the six independent studies. Median estimates of juvenile adjusted ASFs from the multi-window analysis range from 4.5 to 5.5 and mean estimates from 7.1 to 9.4, depending upon the method used to combine studies.

The multi-window and case studies exhibited considerable variability across carcinogens in age-at-exposure related susceptibility. There is also variability in age-at-exposure related susceptibility among studies of the same carcinogen. The sources of variability evident in the analyzed studies include timing of exposure within a given age window, and gender, strain, and species differences in tumor response. The set of studies identified and analyzed in this document was not sufficiently robust to fully describe quantitatively the variability. This variability raises concerns that selection of the median, that is the 50^{th} percentile, estimates for age window-specific ASFs may considerably underestimate effects for certain carcinogens or population groups. Relatively large variability in humans in response to carcinogens is expected to be common (Finkel, 1995; 2002).

Discussion

Taken together, these results indicate that early lifestages are generally more sensitive to carcinogen exposure than adults, and that cancer risk assessment practices should take increased sensitivity of the young into account. When data on age-at-exposure related susceptibility are lacking for a specific carcinogen, these analyses indicate that increased susceptibility of the young is a scientifically justifiable assumption. This early-life susceptibility can be addressed by applying adjustments such as ASFs to the adult cancer potency slope factor when estimating cancer risk associated with early life exposures.

Table 1 illustrates the effect of age-window specific ASFs on lifetime cancer risk. In this example, exposure to the carcinogen is assumed to occur at a constant exposure rate over the entire lifetime. Risk calculations were performed using the mean, 50th, 70th, and 95th percentile ASF values. As shown in Table 1, when increased susceptibility of the fetus, infants, and children is taken into account by applying 50th percentile ASF values, the total lifetime cancer risk is increased two-fold; applying 70th percentile ASF values increases the estimate three-fold, applying mean ASF values increases the estimate nearly five-fold, and applying 95th percentile ASF values increases the estimate 16-fold above the risk estimated in the absence of age-specific adjustments to the potency.

Table 1. Comparison of cancer risk estimates[1] for lifetime exposure to 0.0001 mg/kg-d of a carcinogen with potency 1 (mg/kg-d)$^{-1}$ based on different parameters of ASF distributions[2], or U.S. EPA values.

Age window	Years of life exposed	No adjustment		50th percentile		70th percentile		Mean		95th percentile		U.S. EPA (2005)	
		ASF	Risk	ASF	Risk	ASF	Risk	ASF	Risk	ASF	Risk	Factor	Risk
In utero	0.75	0	0.0	3	3.2×10^{-6}	10	1.1×10^{-5}	21	2.2×10^{-5}	115	1.2×10^{-4}	0	0.0
Birth to <2 yr	2	1	2.9×10^{-6}	13	3.7×10^{-5}	28	7.9×10^{-5}	79	2.3×10^{-4}	350	1.0×10^{-3}	10	2.9×10^{-5}
2 to <16 yr	14	1	2×10^{-5}	5	1.0×10^{-4}	7	1.4×10^{-4}	7	1.4×10^{-4}	20	4.0×10^{-4}	3	6.0×10^{-5}
16 to 70 yr	55	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}
Total lifetime risk			1.0×10^{-4}		2.2×10^{-4}		3.1×10^{-4}		4.7×10^{-4}		1.6×10^{-3}		1.7×10^{-4}

[1] Risk accrued in age window = potency x ASF x exposure rate x (years exposed/70 years).
[2] ASF derived using equal weighting of studies within a chemical (i.e., Method 1 in main text).

Similar, *albeit* more limited conclusions regarding sensitivity of the young to carcinogens were reached by the U.S. Environmental Protection Agency (U.S. EPA, 2005), in its *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens*. Specifically, the U.S. EPA *(*2005) concluded that there is evidence of differential susceptibility for mutagenic carcinogens and recommended adjustments to the adult cancer slope factor and to estimate cancer risk from early life exposure. The U.S. EPA (2005) policy is to determine whether a chemical operates by a mutagenic mode of action, and for those that do, apply a ten-fold adjustment to the adult cancer slope factor for exposures occurring from birth up to two years of age, and a three-fold adjustment for such exposures occurring from 2 up to 16 years of age. The U.S. EPA (2005) does not adjust for increased susceptibility of the fetus to carcinogen exposures, or for the full lifetime ahead for cancer to be manifest following early life exposures. It also does not apply any adjustments for non-mutagenic carcinogens, even though there is increasing appreciation of the importance of epigenetic and other non-mutagenic mechanisms in carcinogenesis, and recognition of epigenetic changes as early events in human carcinogenesis (Baylin, 2005).

The U.S. EPA's factor of 10 for postnatal exposures falls just below the median estimate for the ASF derived for the postnatal window (See Table 1). Thus, while it is consistent with the multi-window analysis presented here, it may result in underestimates of risk for a reasonable fraction of chemicals. The U.S. EPA's factor of three for juvenile exposures is consistent with the range of estimates derived from the multi-window studies, although it falls below the median estimates. It is acknowledged that there are few data available on which to base an estimate for the juvenile period. A factor of three adjusts for the long available time for cancer to manifest when exposure occurs in this period, but would not fully account for inherent differences in susceptibility to cancer, as is observed in breast tissue of pubescent girls exposed to radiation.

The U.S. EPA and existing California practice does not estimate contributions from prenatal carcinogen exposure when estimating lifetime cancer risk. This is an implicit assumption in risk calculation that risk from prenatal exposure is zero. As shown in the multi-window study analysis presented here, this assumption is inconsistent with the available evidence. Moreover, the analysis presented here suggests that a prenatal adjustment factor to the adult potency is

needed; a factor of 10 falls roughly at the 70th percentile for the multi-window studies; the mean value is 21.

Table 1 shows how the application of the U.S. EPA's adjustment factors to calculate lifetime cancer risk compares with the use of the ASF values derived from the multi-window studies here. For example, the use of 70th percentile ASF values as adjustments for the prenatal, postnatal, and juvenile age windows increases the lifetime cancer risk estimate almost two-fold above that estimated using the U.S. EPA's adjustment factors.

Concluding Remarks

OEHHA recognizes the limitations in the data and analyses presented, including limitations associated with the number and types of carcinogens with multi-window exposure data; the non-homogeneous nature of the available multi-window studies; the focus on age windows, without attempting to describe changes in susceptibility within an age window; and the use for several studies of juvenile animals as the later life exposure group in cases where no adult exposure group was included. In addition, in adjusting the ASF to take into account the longer period of time for early carcinogen exposures to manifest, the assumption that the hazard function increases with the third power of age may result in an underestimation of the true sensitivity of these early life stages, if the true rate of increase with age is greater than that.

Still the analyses do provide some guidance on the extent risk may be over- or underestimated by current risk assessment approaches. The analyses support the application of weighting factors to address potential increased susceptibility to carcinogen exposures occurring prenatally and during postnatal and juvenile age periods.

Background

Early-in-life susceptibility to carcinogens has long been recognized by the scientific community and clinicians as a public health concern. Numerous scientific publications and symposia have addressed this issue over the years (e.g., Toth, 1968; Rice, 1979; Napalkov *et al.*, 1989; NRC, 1990; 1993; 1994; Anderson *et al.*, 2000; Miller *et al.*, 2002; Birnbaum and Fenton, 2003; Ginsberg, 2003; Hattis *et al.*, 2004; 2005; Barton *et al.*, 2005). The scientific literature contains a number of human clinical findings and epidemiological studies of early life cancer susceptibility.

Table 2 provides examples of various human cases of early life cancer susceptibility. In the early 1960's, clear cell vaginal adenocarcinoma began appearing in teenagers and young women whose mothers took the synthetic estrogen diethylstilbestrol (DES) to avoid pregnancy loss (Herbst *et al.*, 1971; Preston-Martin, 1989). Observations of marked differences in breast cancer risk in teenage compared to pre-pubescent girls treated for Hodgkin's disease with X-irradiation (Bhatia *et al.*, 1996) underscored the importance of considering life stage in assessing risks of cancer treatment and follow-up to it. The susceptibility of the fetus, infants, and children to thyroid carcinoma following exposure to radioactive iodine (Moysich *et al.*, 2002) and of children under 18 years of age to post-transplant lymphoma (Penn, 2000) has also been recognized.

Table 2. Examples of Early-Life Cancer Susceptibility in Humans

Agent (reference)	Susceptible Group	Case
Diethylstilbestrol (DES) (Herbst *et al*., 1971; Preston-Martin, 1989)	Fetus	*In utero* exposure arising from administration of DES during pregnancy resulted in an increased risk of adenocarcinoma of the vagina and cervix in the daughters, but not in mothers taking the drug
X-Irradiation treatment for Hodgkins lymphoma (Bhatia *et al.*, 1996)	Girls with developing breast tissue (10-16 years old)	10-16 year old girls considerably much more likely to develop breast cancer than those under age 10 similarly treated. Risk of cancer by age 40: 35%
Radioactive iodine fallout from the 1986 Chernobyl accident (Moysich *et al.*, 2002)	Fetus/children	An increased risk of thyroid carcinoma was observed in children from Ukraine and Belarus exposed to radioactive iodine fallout. The greatest risk of thyroid carcinoma was observed in children aged five and under at the time of the accident.
Immunosuppressive drug treatment associated with organ allograft (Penn, 2000)	Children ages 18 years or less	Children are more prone to develop post-transplant lymphomas and lymphoproliferative disorders than adults (53% vs. 15%)

While there are many indications of increased human cancer susceptibility in early life, the magnitude of the impact has been difficult to gauge, and until recently risk assessment procedures have not in general addressed the issue. The California legislature in 2000 recognized the need for a systematic approach to develop scientifically based methods to address this concern so that in environmental decision making special sensitivities of the developing fetus and the young were taken into account. The legislature directed the Office of Environmental Health Hazard Assessment (OEHHA) to assess methodologies used in addressing early-in-life risk, compile animal data to evaluate those methods, and develop methods to adequately address carcinogenic exposures to the fetus, infants, and children (Children's Environmental Health Initiative (AB 2872, Shelly); California Health and Safety Code [HSC] section 901 (a) through (e)).

Here the results of OEHHA's quantitative analyses and synthesis of data from studies in animals exposed to carcinogens during different life stages are presented. First the compilation of data on which the analysis relies is described. This is followed by a description of methods used to analyze the data and derive measures of early-life susceptibility. The analytical approach first evaluates differences in age sensitivity in terms of exposures in different age windows for individuals similarly exposed and followed for similar periods of time – thus it focuses on the inherent susceptibility of the young to the carcinogen. This part of the analysis does not address the longer period of time that those exposed as a fetus, infant, or child have to manifest cancer, also referred to as the longer "shelf-life" (or expected years of life remaining), as compared to those exposed only as adults. Calculations are then presented to address this issue of "shelf-life," the longer period of time for a carcinogenic exposure during youth to manifest compared to an adult exposure. Adjustment factors that would potentially account for early life exposures are then described. These factors would address both the inherent susceptibility to the young to some carcinogens as well as the "shelf life" issue. The work of other bodies or researchers that have suggested or adopted methods to address early life exposure is then described in the context of the analyses and adjustment factors presented here. The document concludes by illustrating the impact of age-window specific ASFs on calculated lifetime cancer risk, assuming in this example that carcinogen exposure occurs at a constant rate across all age windows, from conception through age 70.

Animal Studies of Age Susceptibility

Lifestage Exposure Windows

OEHHA has identified and compiled published animal cancer bioassays exploring age susceptibility issues. Two types of studies with early life exposure were included in this effort. The first type is of studies – "multi-exposure window studies" – that had exposure groups in at least one of the age windows listed below, along with an older age-at-exposure reference group:

- prenatal: from conception to birth
- postnatal: from birth to weaning
- juvenile: from weaning to sexual maturity

An adult exposure group was the preferred comparison or reference group. For the purpose of this analysis, the adult exposure window was defined as the period from sexual maturity to death. A few studies with prenatal or postnatal window exposure groups, did not include an adult group, but did include a juvenile group. In these cases the juvenile group was the reference group used in the data analyses. Studies or groups in which the exposure period for a given group spanned multiple life stages were not included in this effort.

A second type of early life exposure study is used to examine age susceptibility for individual chemicals in depth. These studies support the "case study" of these individual chemicals. For the cases studies, there is at least one group of animals in a bioassay dosed during one of the four exposure windows named above, and there are multiple such bioassays available for each early life exposure window evaluated (e.g., prenatal, postnatal, juvenile). In the current report we present results for two case studies: diethylnitrosamine (DEN) and ethylnitrosourea (ENU).

There is little question regarding whether or not a certain bioassay group should be identified as receiving exposure for certain exposure windows. For example, where exposure to dams ends at birth, offspring can be considered exposed during the prenatal period. The line between the juvenile and adult exposure window is less clear. Assumptions had to therefore be made to categorize exposures used in the bioassays into the exposure windows named above. These assumptions were based on standard reference documents and consultation with developmental biologists and toxicologists. Table 3 gives the ages assumed in establishing the postnatal, juvenile, and adult age windows for the species included in the compiled studies with early life exposure.

Table 3. Age-Specific Exposure Windows by Species[1].

Species	Postnatal: Birth to Weaning	Juvenile: Weaning to sexual maturity	Adult: Sexual maturity/breeding age
Rat ─ male	Day 1-21	Day 22-76	≥ Day 77 (10 wks)
Rat ─ female	Day 1-21	Day 22-55	≥ Day 56 (8 wks)
Mouse	Day 1-21	Day 22-48	≥ Day 49 (7 wks)
Hamster	Day 1-21	Day 22-48	≥ Day 49 (7 wks)
Gerbil	Day 1-28	Day 29-55	≥ Day 56 (8 wks)

[1]The prenatal exposure window is defined as the period from conception to birth for all species. References: Merck, 1998; Harder *et al.* 1993; Fox *et al.*, 1995; Harkness and Wagner, 1995; Charles River, 1999.

Criteria for Study Inclusion

Bioassays examining responses in particular age windows were for the most part designed by different researchers to explore issues related to age susceptibility of carcinogens. The research community did not for the most part standardize protocols for these studies. There is therefore a great deal of variation across studies in terms of dose selection, duration of exposure, number of animals, and length of study duration. To be included in the compilation of studies with early life exposure, a study or an experimental group in a study had to meet minimum requirements.

The criteria for study inclusion are as follows:

- Treated groups were exposed to a single chemical or a single chemical mixture
- Study group was not compromised by severe treatment-related non-cancer toxicity
- Overall the duration of exposure period plus observation period exceeded 40 weeks, unless animals died of tumor
- For included dose groups, study reports age at dosing, age at sacrifice, and site-specific tumor incidence
- Each exposure window treatment group has an appropriate concurrent control group, or, for rare tumors only, an appropriate historical control
- The studies were on mammals.

- Each treatment and control group consists of at least ten animals, unless the conduct and design of the study was well done in all other aspects (e.g., the length of the study was sufficiently long to observe treatment-related tumors) and tumor incidence was high in treated groups and very low in controls.
- Site specific tumor data were reported, and not only total number of tumor bearing animals.
- The test compound was administered in the diet, water, via gavage, or by intraperitoneal (i.p.), intravenous (i.v.), or subcutaneous (s.c.) injection.

For dermal and subcutaneous injection studies, distal tumor findings are utilized (for dermal, other than skin tumors; for injection, non-injection site tumors). While studies designed to histopathologically examine tumors at multiple sites were preferred, studies that examined only a select set of organ/tissue sites were not excluded if the sites examined were known with confidence to be the only target tissues for the chemical and age exposure window in question in that particular strain of animal.

Data Sources

Different approaches were taken to identify animal cancer studies that included groups of animals exposed during early life stages. First, MEDLINE and TOXLINE (National Library of Medicine) databases were searched using combinations of various key words for cancer (e.g., tumor(s), neoplasm(s), cancer, neoplasia, cancerous, neoplasms-chemically induced) and for early-life exposure (e.g., age, age-at-exposure, development (al), prenatal, *in utero*, gestation (al), postnatal, neonatal, juvenile, weaning, weanling, adolescent, adolescence, young). Second, the extensive compilation of bioassays in the *Survey of Compounds which have been Tested for Carcinogenic Activity*, was reviewed. This survey, formerly maintained by the National Cancer Institute as Public Health Service Publication Number 149, or PHS 149, is now available from a private source electronically as CancerChem, 2000. Third, from bibliographies from relevant published papers additional studies were identified. Finally the Single Dose Database developed by Calabrese and Blain (1999) was obtained and utilized to identify additional publications that appeared to contain potentially useful data. All of these publications were evaluated to determine if the study dosed separate groups of animals early in life and at or near adulthood. A

total of 145 publications, providing data on 84 chemicals, were identified as meeting the criteria for study inclusion. A subset of these met the criteria for inclusion in the multi-window analysis.

An Experiment

Here we define an experiment as a study component consisting of a control group as well as a group or groups of animals exposed during the same life-stage exposure window (i.e., prenatal, postnatal, juvenile or adult), and using the same experimental protocol (e.g., route of exposure, strain, species, laboratory). One publication may be a report for multiple experiments.

Multi-Window Studies

Thirty-six of the 145 publications containing studies that met the selection criteria described above reported multi-window studies (Table 3), that is, they included at least one group dosed solely in a defined early life-stage window (prenatal, postnatal or juvenile), a control group and a comparison group of animals exposed only as adults (preferred) or in some cases, juveniles. Thus a multi-window study contains multiple experiments – at least one experiment in a prenatal, postnatal or juvenile exposure window, and another experiment with exposure in an older group, preferably an adult exposure window experiment. The publications on the multi-window studies are listed in Table 4.

As indicated in Table 4, sixteen of the 36 multi-window publications included groups of animals dosed only during the prenatal window, providing data on 14 chemicals. Twenty-five of the multi-window publications included groups of animals dosed only during the postnatal window, providing data on 18 chemicals. Five of the multi-window publications included groups of animals dosed only during the juvenile window, as well as groups of animals dosed only during the adult window, and provided data on five chemicals. Experimental animal species employed in these studies included rats, mice, gerbils, and hamsters.

Table 4. Multi-Window Studies

Chemical, CAS Number	Species	Age Exposure Windows[1]				Publication
		Pr	Po	Ju	Ad	
Benzidine, 92-87-5	Mouse		√	√		Vesselinovitch *et al.*, 1975b
		√	√	√		Vesselinovitch *et al.*, 1979a
Benzo[a]pyrene, 50-33-9	Mouse		√	√		Vesselinovitch *et al.*, 1975a
			√	√		Truhaut *et al.*, 1966
1,1-Bis(*p*-chlorophenol)-2,2,2-trichloroethane (DDT), 50-29-3	Mouse		√	√		Vesselinovitch *et al.*, 1979a
Butylnitrosourea, 869-01-2	Rat	√	√	√		Zeller *et al.*, 1978
Dibutylnitrosamine, 924-16-3	Mouse		√		√	Wood *et al.*, 1970
Diethylnitrosamine (DEN), 55-18-5	Mouse		√	√		Rao and Vesselinovitch, 1973
	Mouse[a]		√	√		Vesselinovitch *et al.*, 1984
	Hamster	√			√	Mohr *et al.*, 1975[e]
		√			√	Mohr *et al.*, 1995
Diethylstilbesterol (DES), 56-53-1	Mouse	√			√	Turusov *et al.*, 1992
7,12-Dimethylbenz[a]anthracene (DMBA), 57-97-6	Rat		√	√	√	Meranze *et al.*, 1969
	Mouse		√		√	Walters, 1966
1,2-Dimethylhydrazine, 540-73-8	Rat		√	√		Martin *et al.*, 1974
Dimethylnitrosamine (DMN), 62-75-9	Hamster	√			√	Althoff *et al.*, 1977
	Rat			√	√	Noronha and Goodall, 1984
Di-*n*-propylnitrosamine (DPN), 621-64-7	Hamster	√			√	Althoff *et al.*, 1977
		√			√	Althoff and Grandjean, 1979
1-Ethylnitrosobiuret, 32976-88-8	Rat	√	√		√	Druckrey and Landschutz, 1971
Ethylnitrosourea (ENU), 759-73-9	Gerbil		√	√		Naito *et al.*, 1985
	Rat		√	√		Bosch, 1977
		√	√	√		Naito *et al.*, 1981
		√			√	Tomatis *et al.*, 1977
	Mouse[a]		√	√		Vesselinovitch *et al.*, 1974
2-Hydroxypropylnitrosamine, 39603-53-7	Hamster	√			√	Althoff and Grandjean, 1979
3-Hydroxyxanthine, 13279-29-3	Rat		√	√	√	Anderson *et al.*, 1978

Table 4. Multi-Window Studies (continued)

Chemical, CAS Number	Species	Age Exposure Windows[1]				Publication
		Pr	Po	Ju	Ad	
3-Methylcholanthrene (3-MC), 56-49-5	Mouse	√			√	Tomatis *et al.*, 1971
			√		√	Klein, 1959
		√			√	Turusov *et al.*, 1973
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 64091-91-4	Mouse	√			√	Anderson *et al.*, 1989
Methylnitrosourea (MNU), 684-93-5	Rat			√[c]	√[d]	Grubbs *et al.*, 1983
	Mouse		√	√		Terracini and Testa, 1970
			√		√	Terracini *et al.*, 1976
β-Propiolactone, 57-57-8	Mouse		√		√	Chernozemski and Warwick, 1970
Safrole, 94-59-7	Mouse	√	√	√		Vesselinovitch *et al.*, 1979a
		√	√	√		Vesselinovitch *et al.*, 1979b
Tetrachlorodibenzodioxin (TCDD), 1746-01-6	Mouse		√[b]	√		Della Porta *et al.*, 1987
Urethane, 51-79-6	Rat	√	√	√	√[e]	Choudari Kommineni *et al.*, 1970
Vinyl chloride, 75-01-4	Rat	√	√		√	Maltoni *et al.*, 1981

[1] Abbreviations: prenatal, Pr; postnatal, Po; Juvenile, Ju; adult, Ad.
[a] Conducted in two strains of mice.
[b]Postnatal dosing extended slightly into the juvenile period.
[c] Dosing initiated toward the end of juvenile period in female rats, from day 50 to 57.
[d]There were two adult female rat exposure groups, one exposed from day 80 to 87, and the other from day 140-147.
[e]Dosing initiated in later part of the juvenile period, from day 46 to 61.

Case Studies Data: DEN and ENU

DEN and ENU are also two well studied model carcinogens, and their modes of carcinogenic action and pharmacokinetic behaviors are relatively well understood. They both are genotoxic, and form DNA adducts; DEN requires metabolic activation, ENU does not. They both cross the placenta. There are numerous experiments on DEN and ENU included in the compilation of studies with early life exposure. For these reasons, these chemicals were selected as case studies. Cancer potencies, defined below, were derived using the data for different age groups. Only data in the mouse were used, as this was the species in which the largest numbers of early life exposure experiments were conducted on DEN and ENU.

DEN. Ten mouse publications on DEN were identified (See Table 5). Among these publications, three included studies of mice exposed during the prenatal window, seven included studies of mice exposed during the postnatal window, and two included studies of mice exposed during the juvenile window. These publications yielded a total of eight prenatal datasets, 18 postnatal datasets, and five juvenile datasets. No "adult only" exposure studies were identified in mice for DEN. Thus the juvenile exposure studies were used as the older age at exposure comparison group. If the juvenile lifestage is more susceptible to DEN exposures than the adult, then the use of these juvenile exposure studies as the comparison group will result in an underestimate of the DEN age ratio distributions for prenatal and postnatal exposure.

Table 5. DEN and ENU Mouse Studies

Chemical, CAS Number	Age Exposure Windows[1]				Publication
	Pr	Po	Ju	Ad	
Diethylnitrosamine (DEN), 55-18-5	√				Anderson *et al.* (1989)
		√			Boberg *et al.* (1983)
		√			Drinkwater and Ginsler (1986)
		√			Lai *et al.* (1985)
	√				Mohr and Althoff (1965)
		√	√		Rao and Vesselinovitch (1973)
		√			Turusov *et al.* (1973)
		√	√		Vesselinovitch *et al.* (1984)
		√			Vesselinovitch (1980)
	√				Vesselinovitch (1983)
Ethylnitrosourea (ENU), 759-73-9		√			Anderson *et al.* (1989)
	√				Diwan *et al.* (1974)
		√			Drinkwater and Ginsler (1986)
	√				Kaufman (1976)
		√			Naito *et al.* (1982)
		√			Pereira *et al.* (1985)
		√			Schmahl (1988)
		√			Searle and Jones (1976)
			√		Vesselinovitch *et al.* (1973)
		√	√		Vesselinovitch *et al.* (1974)
	√				Vesselinovitch *et al.* (1977)
	√	√	√		Vesselinovitch (1983)
	√				Wiggenhauser and Schmahl (1987)

ENU. Thirteen mouse publications on ENU were included in the compilation of studies with early life exposure (See Table 5). Of these, five had studies on mice exposed during the prenatal window, eight during the postnatal window, and three during the juvenile window. These publications yielded a total of 30 prenatal datasets, 27 postnatal datasets, and eight juvenile datasets. As with DEN, no "adult only" exposure studies were identified, and if the juvenile lifestage is more susceptible to ENU exposures than the adult, then the use of these juvenile exposure studies as the comparison group will result in an underestimate of the ENU age ratio distributions for prenatal and postnatal exposure.

Methods

This section describes the methods used to analyze and compare the carcinogenic activities of compounds in different age windows. First a measure of carcinogenic activity, the cancer potency, is defined. Methods for deriving it from animal studies are then described. An age susceptibility factor, or "ASF," measures the carcinogenic activity in an early life-stage exposure window compared to adult exposure. The ASF is defined simply as the ratio of the cancer potency in an early age window to the cancer potency in the adult age window. Cancer potencies and ASFs are estimated from data and not measured precisely. To describe this uncertainty, these measures are described by probability distributions. Methods for the derivation of these distributions are also explained below. For the multi-window studies, ASFs are derived for each experiment, and also for the different chemicals. For the DEN and ENU case studies, cancer potency is derived for each experiment. These in turn are used to derive distributions of cancer potency within a given life stage exposure window. There are no "adult only" exposure experiments for DEN and ENU in mice. The ratios for prenatal to juvenile potencies and postnatal to juvenile potencies are instead derived. Statistical methods employed for this are explained below.

Cancer Potency

Mathematic Model. Cancer potency estimates were derived by applying a linearized multistage (LMS) model to cancer dose-response data from studies in experimental animals. Assuming

dose-response is linear at low doses, the LMS model provides a mechanism of bounding the quantitative estimates of low-dose risk from exposures to carcinogenic agents (Crump *et al.*, 1976; Peto, 1978). The LMS model may be described by the following equation

$$p(d) = 1 - e^{-\sum_{i=0}^{k-1} q_i d^i}, \quad q_i \geq 0, \qquad (1)$$

where p(d) represents the lifetime probability of cancer at a lifetime dose rate, d, and q_i are model parameters that were estimated via maximum likelihood methods, as described below. At low doses the above equation reduces to:

$$p(d) = 1 - e^{-(q_0 + q_1 d)}$$

When q_0 is small this reduces to the simple linear relationship:

$$p(d) = q_0 + q_1 d.$$

where the probability of cancer is represented in the unexposed by intercept q_0 and in the exposed increases linearly with dose d. Here, cancer potency is defined as the parameter q_1: At low doses, it describes quantitatively the extent that cancer risk increases with an incremental increase in dose.

Dose Metric. The work here is to compare cancer potencies from experiments utilizing the same protocols but for the life-stage window in which dosing occurred. The dose metric adopted for this work is the cumulative dose normalized by bodyweight:

$$d = \sum_{i}^{t} d_i$$

d_i, the dose given on i^{th} day of the experiment, is expressed in units milligram amount administered per kilogram animal bodyweight (mg/kg-bw). This results in potencies that are comparable in terms of the initial internal concentration after administration of the compound, and the overall exposure during the life-stage window. The cancer potency q_1 is expressed as the increase in risk with increasing cumulative dose, in units mg/kg-bw.

Experiments did not always report dose administered in units mg/kg-bw. When dose was reported as a concentration administered in diet or water, it was converted to mg/kg-bw based on the amount of food or water consumed, the concentration in the media and the body weight of the animal on the day of dosing. When dose was reported as bulk quantity applied (e.g., mg

amount), it was converted to mg/kg-bw by dividing by the body weight of the animal on the day of dosing.

If the body weight on the day(s) of dosing was not reported in the publication, the default body weight was used. The default body weights of rats and mice were modeled from normative data on common strains of mice (BALB/cANCr, AKR/LwCr, and C57Bl/6Cr) surveyed by Poiley (1972) and rats (Fischer 344 and Sprague-Dawley) surveyed by Poiley (1972) and Cameron *et al.* (1985) using constrained linear regression and the statistical package STATA (Stata Corp, College Station, Texas). The model takes the form:

$$\text{BodyWeight}_{age} = \beta_0 + \beta_1 (\text{day-1}) + \beta_2 (\text{day-1})^2 + \beta_3 (\text{day-1})^3 + \beta_4 (\text{day-1})^4 ,$$

where β_0 was defined as the measured average body weight on day 1 of life (i.e., redefining day 1 as 'day 0' or the origin). The variable day is the day of life, and parameters, β_1, β_2, β_3, β_4 are estimated. Fitted values for each day of life from birth through six months of age (i.e., day 168) for male and female mice (applied to all strains) and male and female rats (separate body weight tables are given for Sprague-Dawley rats and all other strains) are provided in Appendix A.

Procedure to Estimate Cancer Potency

Model parameters were estimated using maximum likelihood methods, using a forward selection process. The forward selection process commences with the data being fit to a 2-parameter LMS model. If the goodness-of-fit test indicates an adequate fit (at the $p = 0.05$ level) then the 2-parameter LMS model is used to compute the cancer potency. If the 2-parameter model does not satisfactorily fit the data, a 3-parameter model is fit. This model is then assessed via a goodness-of-fit test. The process of adding an additional parameter and assessing model fit continues until the goodness-of-fit statistic is no longer statistically significant.

In some cases the dose response data are not consistent with an upward curving dose response relationship, such that tumor incidence can initially increase with dose and then remain steady or decrease as doses are further increased. This can occur from competing causes of mortality such as cancers at sites other than the one being analyzed, and other causes of death. It can also result from pharmacokinetics for example when a chemical requires activation for carcinogenic

activity, and the activation pathway saturates as dose is increased. Following the inclusion criteria described above, when mortality from noncancer toxicity is high, the study is not suitable for inclusion in the data base. There are a few datasets included in these analyses where, despite meeting the study inclusion criteria, the LMS does not fit the data well. For these datasets, a procedure laid out in Anderson *et al.* (1983) is used to remove high dose groups. Working down from the highest dose group, dose groups are removed, the model fitted, until there is an adequate fit of the model to the data (goodness-of-fit, $p > 0.05$).

The analysis begins by focusing on experiments conducted for a given age window, and deriving cancer potency estimates for each experiment conducted with groups in that age window.

The method of maximum likelihood is implemented to obtain the model parameter estimates for each experiment. Here the parameter of greater interest is the potency, q_1, the slope term in equation (1). The idea behind maximum likelihood parameter estimation is to determine the parameters that maximize the probability (likelihood) of observing the sample data. For each animal, the probability of cancer is given by equation (1). Assuming each animal within an exposure window exposed to dose d_i has the same chance of developing cancer at a specific site (or arising from a specific cell type), the probability of observing r_i animals with that cancer out of n_i animals total may be described by the following binomial distribution,

$$\binom{n_i}{r_i}[p(d_i)]^{r_i}[1-p(d_i)]^{(n_i-r_i)}. \qquad (2)$$

For a given experiment, there are different dose groups, that is d_i is the same for each animal within the dose group, but differs across the dose groups. The likelihood is constructed by assuming that animals across the dose groups are independent, and the likelihood is the product of the term (2) above across the k dose groups or categories, i.e.,

$$L([q_0,q_1,\ldots,q_{k-1}]) = \prod_{i=0}^{k-1}\binom{n_i}{r_i}[p(d_i)]^{r_i}[1-p(d_i)]^{(n_i-r_i)}. \qquad (3)$$

The support function, also referred to as the log-likelihood, is defined as the natural logarithm of the likelihood function (3), disregarding constants, i.e.

$$S([q_0, q_1, \ldots, q_{k-1}]) = \ln L([q_0, q_1, \ldots, q_{k-1}])$$

$$= \sum_{i=0}^{k-1} r_i \ln[p(d_i)] + (n_i - r_i)\ln[1 - p(d_i)]. \qquad (4)$$

The values of q_0, q_1, . . . , q_{k-1} that maximize equation (4) are the maximum likelihood estimates. Profile-likelihood methods are used to trace the likelihood to determine the 0.5% through the 99.5% (in increments of 0.5%) confidence bounds of the linear slope parameter of the model, q_1. This is done to describe the uncertainty in the estimates of this parameter, as well as the confidence we may have that the parameter falls below some upper bound value. Determining the confidence percentiles of the slope parameter q_1 provides a discretized distribution of this parameter.

The above procedure is performed for each treatment related tumor site or type in the experiment, that is for each site or type for which a treatment-related increase in tumors has occurred (i.e., a statistically significant increase in tumor response in the exposed compared to the treatment group [$p \leq 0.05$], or a biologically significant finding of rare tumor). For studies in which a carcinogen causes tumors at multiple sites or of multiple types, a combined "multisite" potency distribution is estimated from the site/type-specific potency distributions. A combined distribution of cancer potency is created by statistically summing across the site/type specific potency distributions for each treatment-related tumor site/type in the experiment, using a Monte Carlo procedure with 100,000 iterations per experiment. In performing this analysis the cancers at the different sites/types are assumed to be independent. The result of this procedure is an estimate of potency for the total treatment caused cancer burden observed in the experiment.

In a given experiment, not all groups were observed for the same length of time. Therefore in computing potency for a given exposure window within a study, all observation periods were normalized to the same time length (t_{obs}), typically the observation period for the control animals. For the purpose of this calculation the observation period is defined as the time between the age at the initiation of dosing (t_d) and the age the animals were killed (t_m). Following the National Toxicology Program (Bailer and Portier, 1988), cancer was assumed to increase with the third power of age and an adjustment $(t_m - t_d)^3 / t_{obs}^3$ was applied to each group. In cases where all

groups were observed for the same period, adjustment was not necessary. For the case studies, all potency distributions were adjusted to a two year observation period.

Age Sensitivity Factor

Cancer potency is derived for each experiment, which again consists of groups of animals (e.g., all dosed within the same defined exposure window (i.e., prenatal, postnatal, juvenile, or adult), and following a similar experimental protocol but for dose level. In some cases different groups of animals were dosed at the same level (e.g., on a mg/kg-bw basis) on different days within the same exposure window (e.g., postnatal day 1 vs. postnatal day 15). If tumor incidences were not statistically significantly different (at the p = 0.05 level) between the groups dosed on different days within the same exposure window, the incidence data from the groups were combined. If a statistically significant difference was observed, then each of the groups was treated as a separate experiment. For each exposure window, a potency distribution is obtained for each experiment conducted. The cancer potency from "early life" exposure was compared to that from "later life" exposure. This comparison is facilitated by taking the quotient of the cancer potency distribution for those animals exposed in early life and those animals exposed in later life. This ratio distribution for multi-window studies is termed the age sensitivity factor (ASF) distribution. For example the *ASF* for the prenatal exposure window is given by:

$$ASF = q_{1prenatal} \div q_{1\ adult}$$

The dividend is the cancer potency distribution for the prenatal exposure window $q_{1prenatal}$ and the divisor is the cancer potency distribution for the adult exposure window $q_{1\ adult}$. Thus, the quotient distribution represents the spectrum of cancer induction sensitivity in an early-life exposure window relative to adults (or, in some instances juveniles when adult data are not available).

Of particular importance is the location of the ASF distribution in relation to the reference value of 1.0. An ASF distribution that primarily lies above the value of 1.0 indicates early life exposures to a carcinogen result in a stronger tumor response relative to adult exposure. Conversely, an ASF distribution that mainly lies below the value of 1.0 indicates early life exposure to a carcinogen results in a weaker tumor response relative to adult exposure.

Deriving ASF for Multi-Window Studies

For each early-exposure window, ASFs are derived for each study with experiments for which a chemical was administered during that exposure window. There are multiple studies on different chemicals for each exposure window. These different chemical carcinogens act by a variety of mechanisms, for which ASF distributions were determined by the above described methods. Combining these ASF distributions across all chemicals within an "early life" exposure window, results in a description of the magnitude and variability of age at exposure effects for the studies analyzed on these different chemicals. This provides a means by which the susceptibility of that age window to carcinogen exposure relative to the adult may be characterized for the data analyzed. A single "ASF mixture distribution" for each early-life exposure window is derived via Monte Carlo re-sampling methods across all of the chemicals representing a given exposure window. This ASF mixture distribution for a particular exposure window describes the variability in the ASF across these chemicals, and the uncertainty in the ASF. To derive the mixture distribution, each chemical in the data set is equally likely to be sampled, and each chemical is represented by a single ASF distribution. When there are multiple ASFs (representing multiple studies) on a chemical, three different methods are used to sample from them to derive the ASF mixture distribution for the chemical.

Method 1 − Each of the ASF distributions are equally likely to be selected.

Method 2 − Each of the ASF distributions is sampled based upon an inverse-variance weighting scheme. In this case, the variance is calculated for the distribution of the logarithm of the ASF, Var[logASF]. The likelihood that an ASF is sampled is proportional to 1/Var(log[ASF]). The variance of the logarithm of q_1 is used because potencies tend to differ by factors rather than in a linear fashion.

Method 3 − The ASF distribution with the largest median is used as the representative "mixture" ASF distribution to represent the chemical.

Once an ASF distribution is derived for each chemical by one of these methods, these distributions are used to derive the ASF for the group of chemicals. For each chemical, an ASF value is randomly chosen from its ASF distribution. This process proceeds for each of the

chemicals in the group and is replicated 1,000,000 times to derive an ASF mixture distribution for each early-life exposure window. To evaluate the robustness of the study findings, ASF mixture distributions were generated using each of the three methods described above.

Chemical-Specific Case Studies

Methods to compare early vs. later life cancer potencies for the DEN and ENU case studies proceed differently from multi-window studies. For each case study there are several experiments within each exposure window. Experiments for the adult exposure window are not available for either of these chemicals. For these chemicals prenatal and postnatal cancer potencies are compared to juvenile cancer potencies.

For each chemical, an overall distribution of the logarithm of potencies is created for each life stage exposure window. This is accomplished via Monte Carlo methods to sample from each of the individual potency distributions derived for each experiment for that exposure window. Values are sampled from these different potency distributions to create an overall potency distribution for that exposure window. Overall potency distributions for the different exposure windows are used to create a distribution of the ratio of the prenatal to juvenile potencies, and similarly for the postnatal to juvenile potencies.

To test the sensitivity of the result to different assumptions, different sampling weighting schemes are used to create the potency distribution for an age window:

Method 1 − For a given lifestage exposure window, each (log) distribution derived from an individual experiment was equally likely to be selected.

Method 1 (truncated) − A variation of Method 1 was also employed in which each individual potency distribution was truncated at the fifth and ninety-fifth percentiles prior to creating the equally weighted potency mixture distribution. The rationale for truncating each of the potency distributions prior to creating the mixture distribution is to eliminate the most extreme values from each potency distribution.

Method 2a − The potency distributions were sampled based upon weights equal to the computed inverse-variance of each (logarithm) potency distribution. That

is, the variance is calculated for the distribution of the logarithm of the q_1, Var[log q_1]. The likelihood that an q_1 is sampled is proportional to 1/Var(log[q_1]).

Method 2b − The potency distributions were sampled based upon weights equal to the computed interquartiles (25^{th} ($q_{1\ 25}$) to 75^{th} ($q_{1\ 75}$) percentiles) of each (logarithm) potency distribution. The likelihood that an q_1 is sampled is proportional to $1/(\log(q_{1\ 75}) - \log(q_{1\ 25}))$.

By using one of these methods, a potency mixture distribution for each exposure window is obtained. The ratio of mixture potency distributions for a given prenatal or postnatal exposure window to the potency distribution for the juvenile exposure window is computed to arrive at the ratio distribution for that early life stage. In general, exposures during the juvenile age window are expected to result in greater sensitivity to carcinogens than adult exposures, thus the ratios calculated here should be considered underestimates of the true ASF (i.e, the ratio of early to adult potencies) for these chemicals.

Age at Exposure ("Shelf-Life") Adjustments

The ASF and age window sensitivity ratios described thus far address the inherent susceptibility of the young compared to older animals to the carcinogen. The exposures were for individuals similarly exposed and followed for similar periods of time. These ratios do not address the longer period of time the carcinogenic insult to the young has to manifest, also referred to as the longer "shelf-life" (or expected years of life remaining) of the carcinogen-exposed fetus, infant, or child, as compared to the shorter "shelf-life" of the carcinogen-exposed adult. The following provides an adjustment to address the longer period of time for a carcinogenic exposure during youth to manifest compared to an adult exposure.

Here an adjustment is developed based on the Armitage and Doll (1954) mathematical description of carcinogenesis. This approach has been applied in various contexts to consider the impact of dosing and observation at different ages (see e.g., Murdoch *et al.*, 1992; Crouch, 1983; and Crump and Howe, 1984). The model assumes that cancer derives from a single cell after it has undergone a series of transformations. While there have been numerous scientific developments advancing the understanding of carcinogenesis since Doll and Armitage first published their model, the model nonetheless provides a good statistical description of age

dependent observations of cancer development. Thus, this is the context in which the model is applied here.

Assumptions are required for the application of the Doll-Armitage model regarding: 1) the mathematical relationship between applied dose and the probability that a "stage transition" has occurred, 2) the stage affected by the carcinogen and 3) the number of "stages." For the particular forms used to fit the tumor data in this report, a linear relationship is assumed between dose and cell transformation, and the carcinogen is assumed to affect an early stage of the cancer process.

If the probability per unit time of the stage transformation depends linearly on dose rate (*d(t)*), and the carcinogen only affects a single "stage," the probability of tumor by time T_e becomes

$$P(T_e) = 1 - \exp[-(A + BD)] \qquad (5)$$

with

$$D = \frac{1}{T^m \cdot \beta(m-j+1, j)} \int_0^{T_e} d(t)(T_e - t)^{m-j} t^{j-1} dt \qquad (6)$$

where T_e is the time to observation, and β is Euler's beta function (see Crouch, 1983; Murdoch *et al.*, 1992). Here the adjustment is developed for analyses in rodents, so the default lifetime of the test animal is assumed. Following Anderson *et al.* (1983) this is two years for rats and mice. The integer *m* (the number of "stages") specifies the rate of increase in incidence with time and *j* is the "stage" affected by the carcinogen. To adjust for less than lifetime experiments in estimating cancer potency, the hazard function is assumed to increase with the third power of age, corresponding to a value for *m* of 3.0. This is consistent with the poly-3 correction used by the National Toxicology Program in statistical analyses of tumor data in rodent cancer bioassays (Bailer and Portier, 1988). The chemicals here demonstrably act at an early stage, and it is assumed therefore $j = 1$. the solution to Equation 6 describing the constant daily dose (D) equivalent to a daily dose *d* given over a time interval from *a* to *b* becomes, for $j = 1$ and $m = 3$:

$$D = d \cdot \left[\frac{(T_e - a)^3 - (T_e - b)^3}{T^3} \right]. \qquad (7)$$

The intervals used to calculate the adjustment factors for each of the three early age windows are: the day of birth for the prenatal window and from birth to age 21 days for the postnatal

window. The juvenile and adult multi-window studies are in the rat; the interval used for the adjustment is age 22 to 65 days, with 65 days being the midpoint between sexual maturity for the female and male rats. Inserting these intervals into Equation 7, and comparing the result with the average lifetime daily dose associated with dosing in that age interval provides the adjustment factor. The adjustment factors for the prenatal, postnatal and juvenile windows are 3, 2.9 and 2.7, respectively.

Results

Here we present the results of analyses of data from the multi-window studies listed in Table 4 and from the case studies in mice of DEN and ENU listed in Table 5. In the case of the multi-window analyses, ASF distributions derived from individual studies within each early life exposure window are presented, as well as prenatal, postnatal, and juvenile mixture ASF distributions representative of those for the chemicals studied in each of these early life windows. For the DEN and ENU case studies, cancer potency distributions for each of the individual experiments are presented, and then potency ratio distributions, representing the ratio of prenatal to juvenile potency, and the ratio of postnatal to juvenile potency. These ratios are derived as distributions, representing the uncertainty in potency and variability in sensitivity of the animal strains on which these potencies are based.

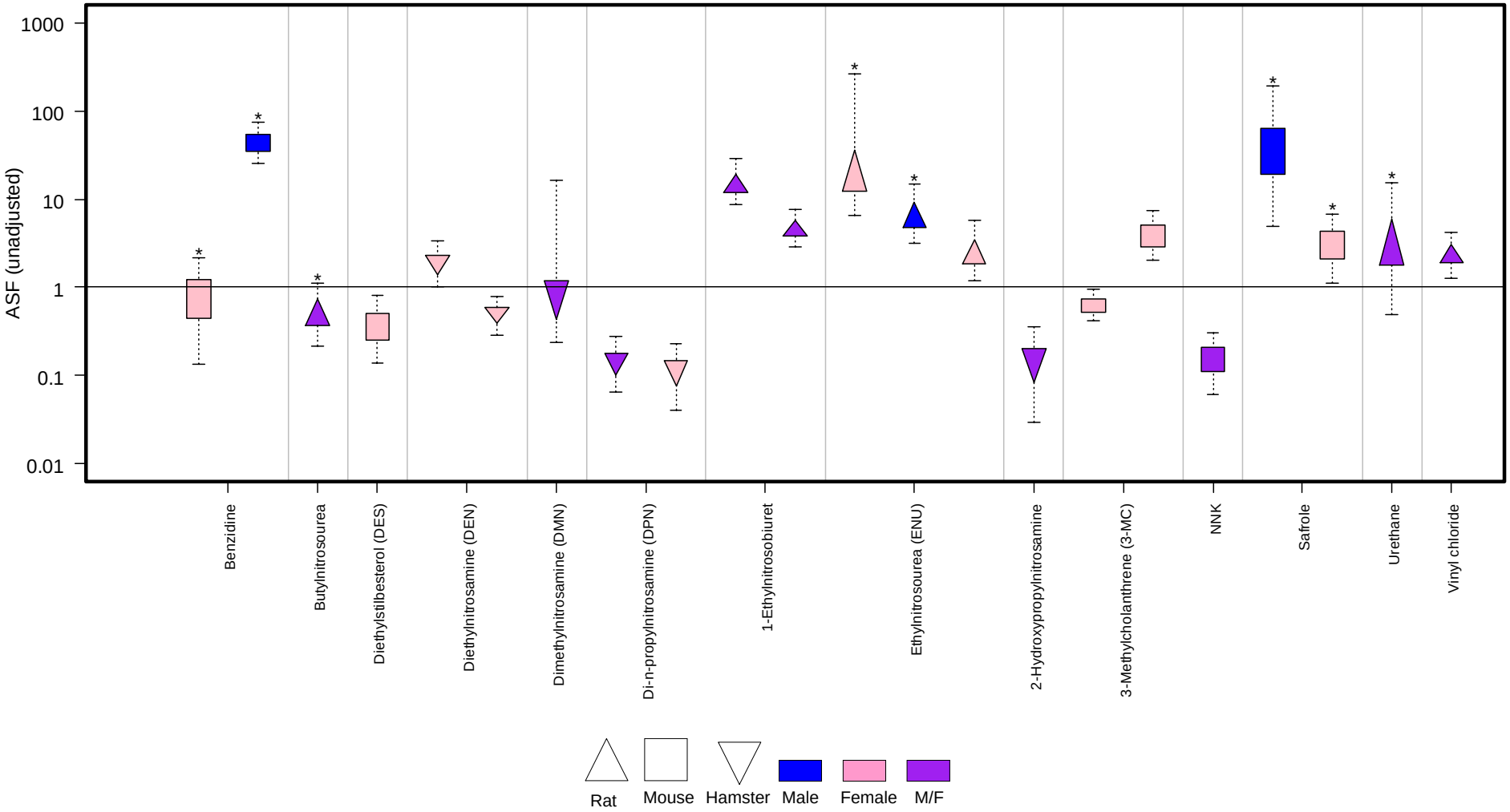
Prenatal Multi-Window Studies

Prenatal Study Specific ASFs

Prenatal ASF distributions were generated for each of 22 multi-window prenatal studies extracted from the 16 publications with prenatal exposure groups listed in Table 4. Fourteen unique carcinogens are covered. Six of the 14 chemicals have two datasets representing each chemical and one chemical, ENU, has three. Figure 1 displays the prenatal ASFs for these studies. They are plotted on a logarithmic scale as "box plots," with upper 75th and lower 25th percentiles as the upper and lower edges of the boxes and triangles, and the upper 95% and lower 5% bounds as horizontal marks above and below the edges of the box. Appendix B, Table B1,

gives the numerical values for these bounds, along with the mean and median for each of the displayed distributions.

Figure 1. Prenatal ASF Distributions



(*ASF calculation is based on juvenile potency distribution)

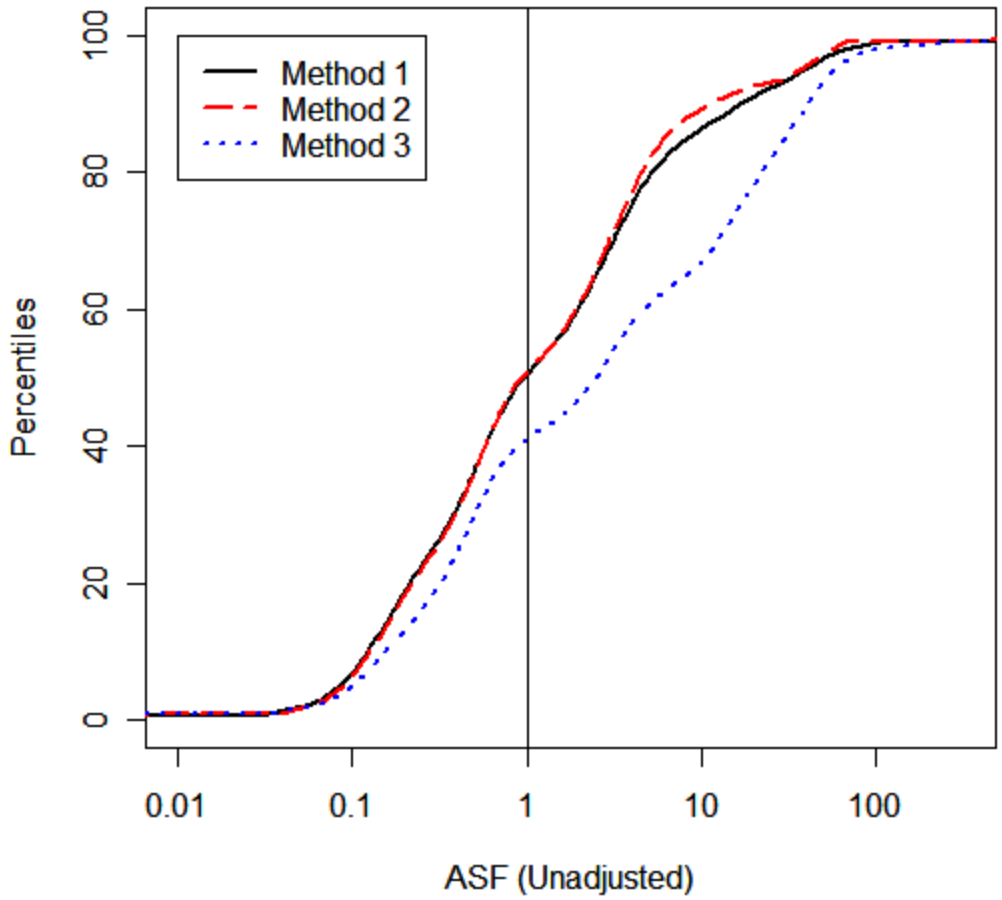
Considerable variability in prenatal sensitivity is evident for the 14 carcinogens, with several demonstrating an enhanced tumor response, a few indicating an equivalent response, and others demonstrating a reduced tumor response associated with prenatal exposure as compared to adult exposure. The prenatal ASF 90% confidence intervals included values less than 0.1 for di-n-propylnitrosamine (based on studies in hamsters), 2-hydroxypropylnitrosamine (hamsters), and NNK (mice), values greater than 10 but less than 100 for benzidine (male mice), 1-ethylnitrosobiuret (rats), ENU (male rats), and urethane (rats), and values greater than 100 for ENU (female rats) and safrole (male mice). Twelve of the prenatal ASF distributions, representing studies of eight carcinogens, had medians that exceed unity. The remaining ten distributions, representing studies of nine carcinogens, had medians that were less than one.

Prenatal ASF Mixture Distributions

The ASF mixture distributions characterize and summarize the prenatal ASF distributions from the multi-window prenatal studies displayed in Figure 1. As described in greater length in the Methods section above, in these derivations each chemical was given equal weight and three different methods were used to obtain the ASF mixture distribution.

Figure 2 displays the prenatal ASF mixture cumulative distribution functions determined via Methods 1 through 3. For all three methods, these prenatal ASF distribution functions are essentially bimodal, with significant portions of each of the distributions below and above 1.0.

Figure 2. Prenatal ASF Mixture Cumulative Distribution Functions



The mean, and specific percentiles for each method are provided in Table 6. For the 30th percentile and below there is essentially no difference between the ASF distributions across the methods. Slight differences between Method 1 and Method 2 appear at the latter percentiles, at the 80th percentile and greater. For percentiles greater than the 30th, the prenatal mixture ASF distribution derived via Method 3 has percentile values that are larger than the other methods. The distribution derived via Method 1 falls between Methods 2 and 3. These prenatal ASF mixture cumulative distribution functions follow a predictable pattern that is explained via the mixing algorithms employed. The distributions generated by each of the three methods are multimodal with modes above and below unity (Figure 2). The multimodal nature of the distributions is clearly illustrated by the mixture frequency distributions, which are presented and discussed in more detail in Appendix C.

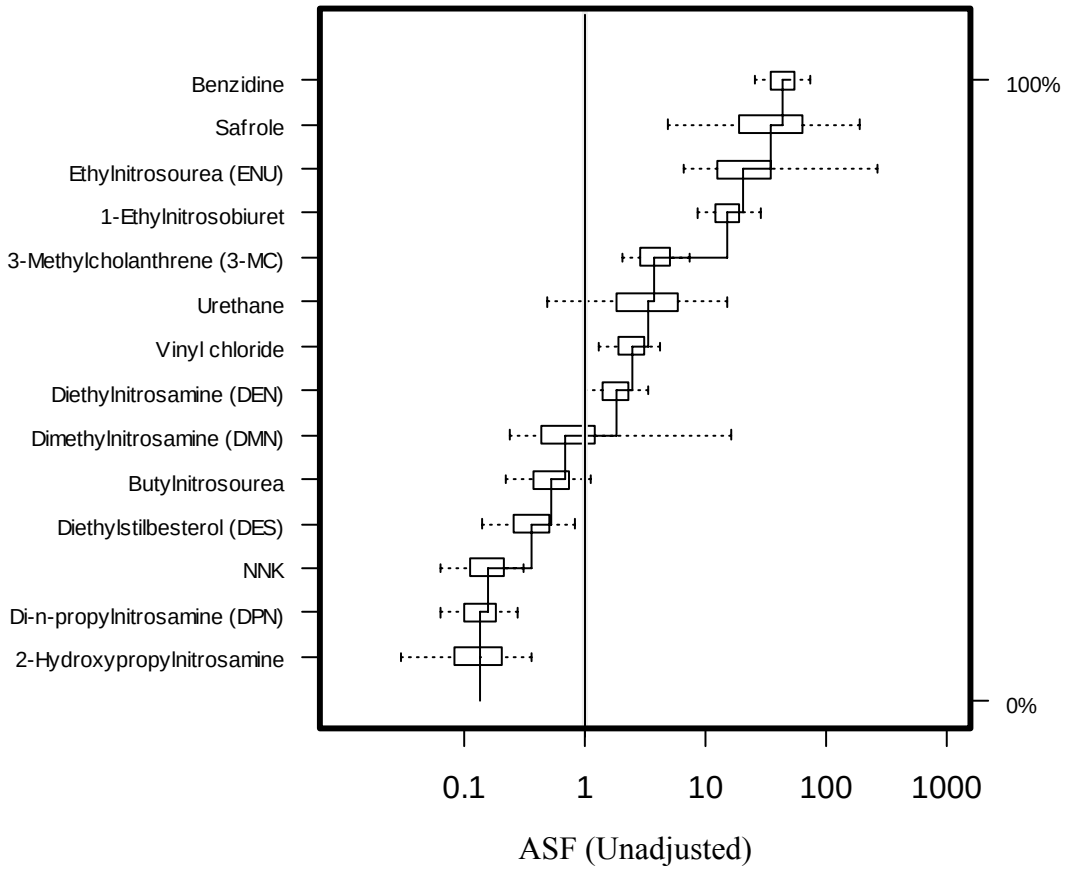
Table 6. Prenatal ASF Mixture Distribution Statistics by Method

Statistics	Unadjusted			Adjusted		
	Method 1	**Method 2**	**Method 3**	**Method 1**	**Method 2**	**Method 3**
Mean*	7.03	5.54	13.73	21.09	16.62	37.07
Percentiles						
5th	0.09	0.09	0.10	0.27	0.27	0.30
10th	0.12	0.13	0.15	0.36	0.39	0.45
20th	0.22	0.22	0.32	0.66	0.66	0.96
30th	0.38	0.39	0.50	1.14	1.17	1.50
40th	0.58	0.58	0.89	1.74	1.74	2.67
50th	0.96	0.93	2.49	2.88	2.79	7.47
60th	1.95	1.92	4.68	5.85	5.76	14.04
70th	3.11	2.96	12.39	9.33	8.88	37.17
80th	5.18	4.57	22.11	15.54	13.71	66.33
90th	16.52	11.18	40.35	49.56	33.54	121.05
95th	38.49	36.15	57.28	115.47	108.45	171.84

* Calculated excluding large values above the 99th percentile.

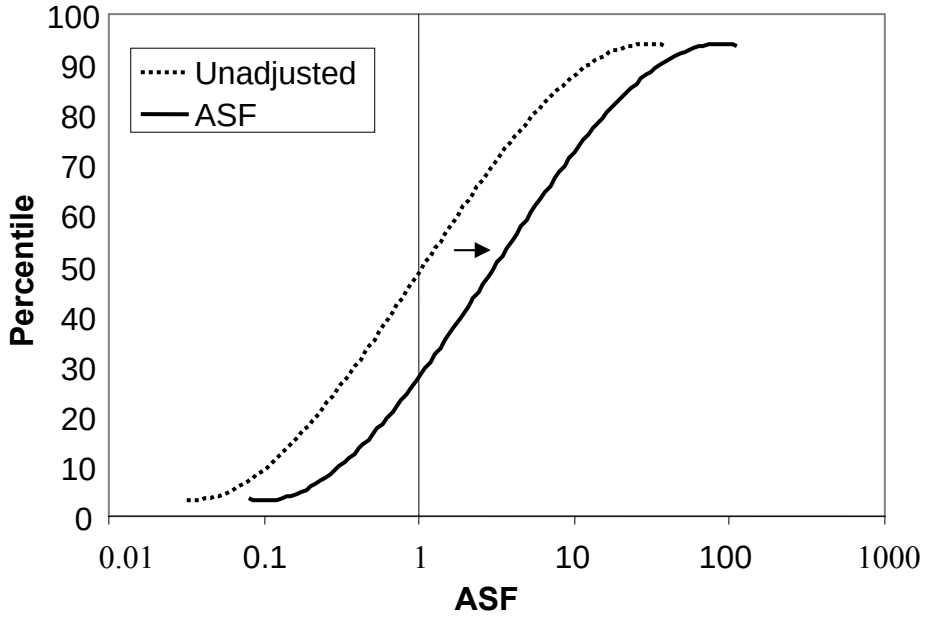
Figure 3 shows the individual prenatal ASF 90% confidence bounds for each of the datasets used in generating the prenatal ASF mixture frequency distribution where a single dataset was taken as representative of each chemical using Method 3. The ASF 90% confidence bounds are displayed as a cumulative frequency profile.

Figure 3. Prenatal ASF Cumulative Frequency Profile



To summarize, the inherent sensitivity of animals to *in utero* exposures to the carcinogens examined here appears dependent on the carcinogen and the animal species, sex, and strain, as is indicated in the curve showing unadjusted ASFs (Method 1) in Figure 4 below. For some chemicals, the animals were less susceptible *in utero* compared to adult exposure, and for a number of other cases just the opposite was observed. Once an adjustment is made for timing of exposure, the majority of cases indicate greater susceptibility early in life, with a fraction of cases showing substantial sensitivity (Figure 4).

Figure 4. Method 1 Prenatal ASF Mixture Distribution

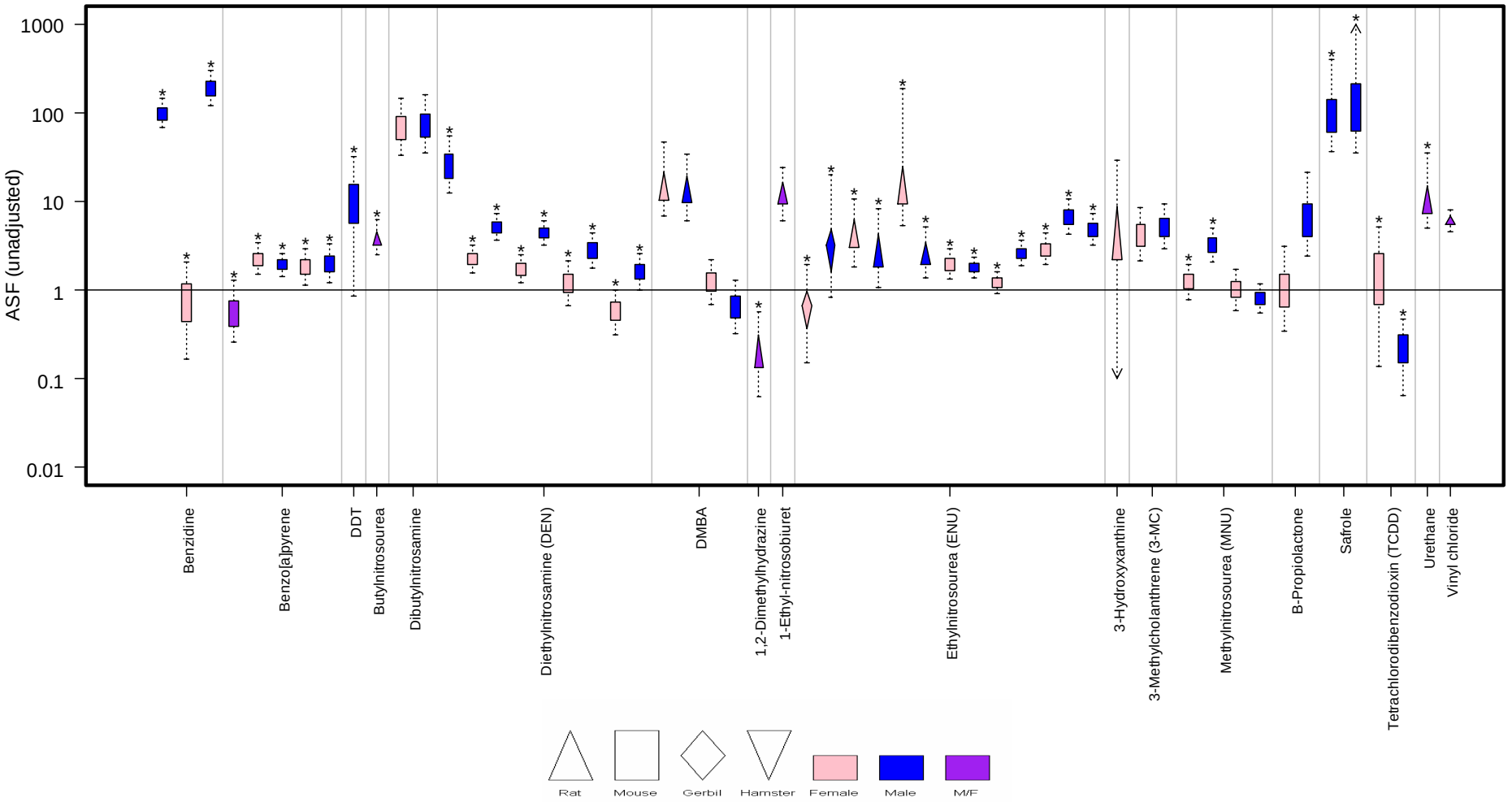


Postnatal Multi-Window Studies

Postnatal Study Specific ASFs

Postnatal ASF distributions generated for each of 55 multi-window studies are displayed in Figure 5. These studies were extracted from the 25 publications listed in Table 4 that included a postnatal exposure group. Eighteen unique carcinogens are represented. Eleven of the 18 chemicals have two or more datasets representing them. As for the prenatal case, Figure 5 displays the ASFs for these studies as box plots. Mean, 5^{th}, 25^{th}, 50^{th}, 75^{th}, and 95^{th} percentile values for each of the ASF distributions are given in Appendix B, Table B2.

Figure 5. Postnatal ASF Distributions



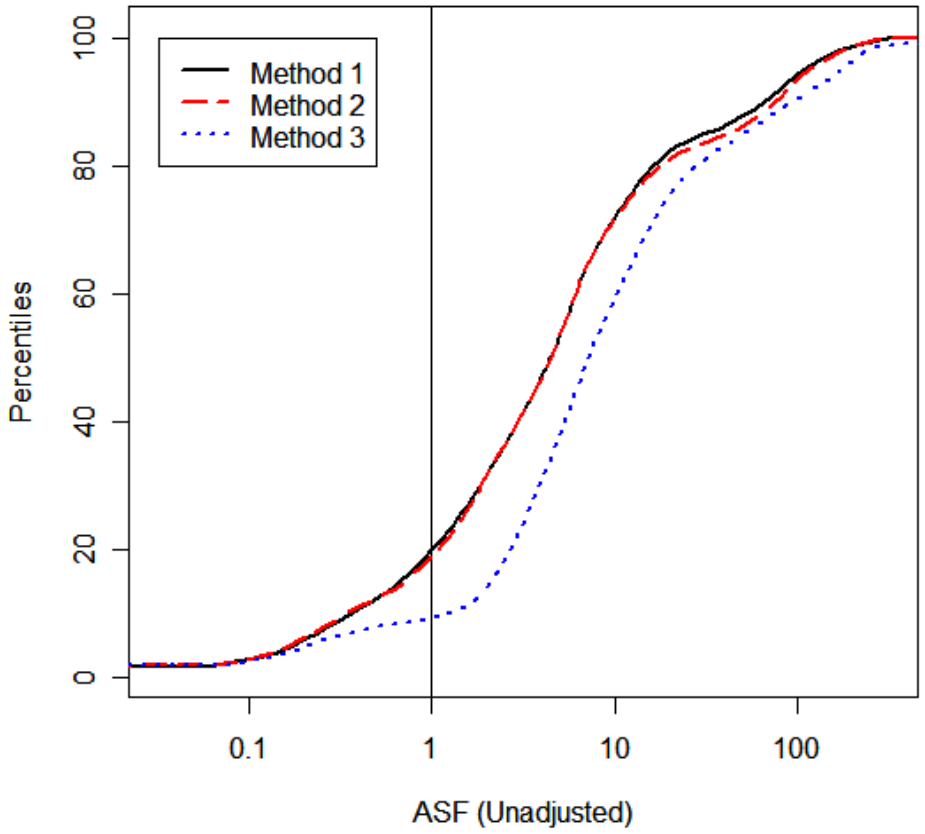
(*ASF calculation is based on juvenile potency distribution)

For two-thirds of the studies plotted - thirty-seven postnatal datasets (for 15 carcinogens) – the ASFs are significantly greater than unity (i.e., the lower 95% confidence bound exceeds unity). For sixteen postnatal studies or 29% of the total, representing nine carcinogens, 90% confidence intervals straddle unity. Two postnatal studies, or only 4% of the plotted studies, representing two carcinogens, have ASFs with upper 95% confidence bounds less than unity.

Postnatal ASF Mixture Distributions

Figure 6 displays the postnatal ASF mixture cumulative distribution functions determined via Methods 1 through 3 described above.

Figure 6. Postnatal ASF Mixture Cumulative Distribution Functions



The postnatal ASF mean, and certain percentiles for each method are provided in Table 7. The cumulative distribution functions for Method 1 and Method 2 are nearly identical up to the 70th percentile. After the 70th percentile, Method 2 has slightly larger values as compared to Method 1. The most compact postnatal ASF distributions generally have values that are significantly greater than unity. As a result, the inverse-variance method (Method 2) produces a mixture cumulative distribution that is shifted slightly to the right of the distribution derived using

Method 1, where equal weighting is given to all studies within a chemical. The magnitude of this rightward shift with Method 2 is not particularly large however because there were no single studies amongst those chemicals with multiple studies with considerably smaller variances than the others in the set. The postnatal ASF mixture cumulative distribution derived via Method 3 has percentile values that are considerably larger than the other methods beyond the 5th percentile. The most peaked mode of the postnatal ASF mixture frequency distribution is similar across the mixing algorithms employed (i.e., Methods 1-3). However, when a single study with the largest median value is selected to represent the chemical (Method 3), the percentiles of the distribution become somewhat larger as compared to that seen using Methods 1 or 2. More details on the results for the postnatal multi-window analysis by the three methods are given in Appendix C.

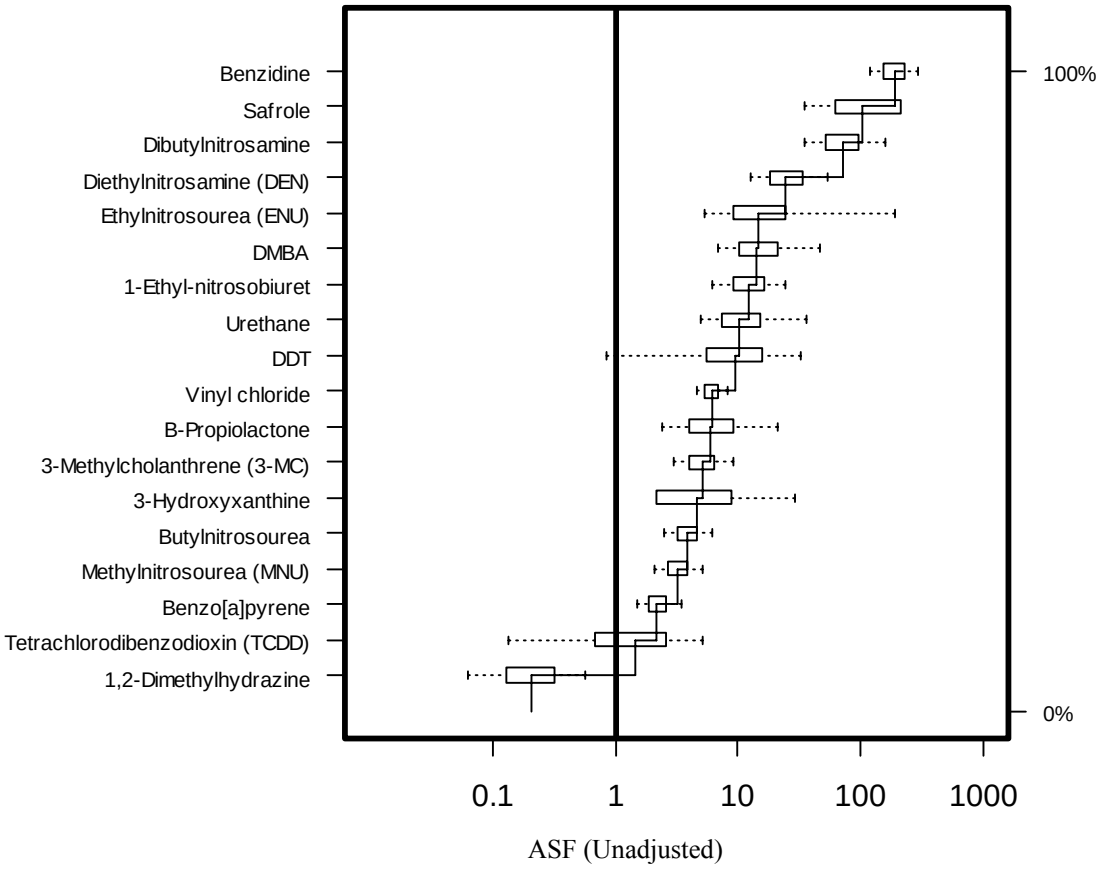
Table 7. Postnatal ASF Mixture Distribution Statistics by Method

Statistics	Unadjusted			Adjusted		
	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3
Mean*	27.08	27.62	42.45	78.53	80.10	123.11
Percentiles						
5th	0.20	0.20	0.26	0.58	0.58	0.75
10th	0.41	0.40	1.48	1.19	1.16	4.29
20th	1.08	1.14	2.80	3.13	3.31	8.12
30th	1.93	1.94	4.01	5.60	5.63	11.63
40th	3.13	3.10	5.54	9.08	8.99	16.07
50th	4.64	4.61	7.45	13.46	13.37	21.61
60th	6.35	6.29	11.00	18.42	18.24	31.90
70th	9.62	9.60	16.99	27.90	27.84	49.27
80th	18.10	19.71	33.58	52.49	57.16	97.38
90th	72.78	81.79	106.08	211.06	237.19	307.63
95th	122.82	129.22	188.14	356.18	374.74	545.61

* Calculated excluding large values above the 99th percentile.

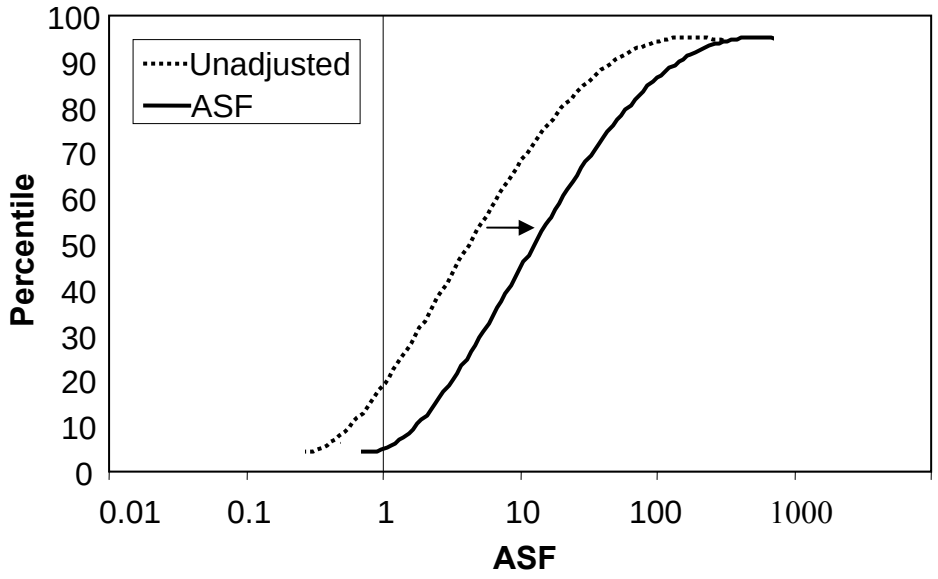
Figure 7 shows the individual prenatal ASF 90% confidence bounds for each of the datasets used in generating the postnatal ASF mixture frequency distribution where a single dataset was taken as representative of each chemical (Method 3). The ASF 90% confidence bounds are displayed as a cumulative frequency profile.

Figure 7. Postnatal ASF Cumulative Frequency Profile



To summarize, in general for the cases studied here animals are inherently more sensitive in the postnatal period, as indicated by the unadjusted ASF (Method 1) shown in Figure 8 below. Once an adjustment is made for timing of exposure, the difference between possible contributions of early postnatal versus adult exposures becomes more pronounced, as indicated in Figure 8 by the ASF curve.

Figure 8. Method 1 Postnatal ASF Mixture Distribution



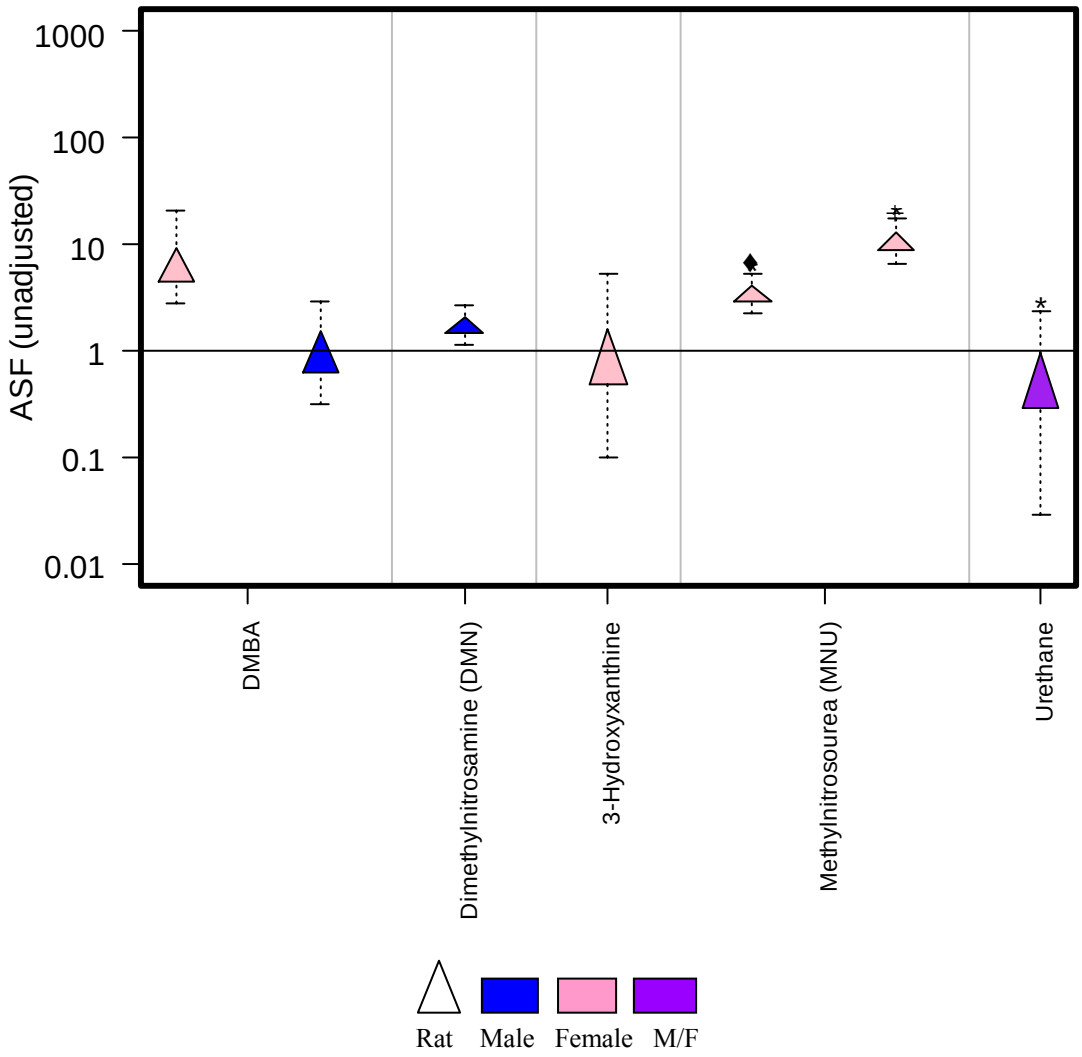
Juvenile Multi-Window Studies

Juvenile Study Specific ASFs

Juvenile ASF distributions were generated for each of seven multi-window studies extracted from five publications with juvenile and adult exposure groups, covering five unique carcinogens (See Table 4). Figure 9 displays the juvenile ASFs in boxplot form. Appendix B, Table B3, provides the mean, 5^{th}, 25^{th}, 50^{th}, 75^{th}, and 95^{th} percentile values for each of these ASF distributions. All studies were conducted in rats. Four studies have juvenile ASFs significantly greater than unity ($p \leq 0.05$), and the 90% confidence interval straddles unity for the remaining three studies. Of the two ASF distributions representing the chemical MNU from the publication of Grubbs *et al.* (1983), only one is used in determining the juvenile ASF mixture distribution, since the two ASF distributions are not independent. The juvenile exposure data (representing the numerator of both ASF distributions) are from the same group of female rats exposed on days 50 through 57, but the adult exposure data (representing the denominators of the ASF distributions) differ. In the first MNU juvenile ASF distribution the adult exposure data are from females exposed on days 80 through 87. In the second MNU juvenile ASF distribution the adult exposure data are from females exposed on days 140 through 147. These MNU data illustrate that even within the adult lifestage, the earlier the exposure occurs, the more sensitive the animal

is to the carcinogen (i.e., MNU-induced mammary tumors). For DMBA, the juvenile females are significantly more sensitive than the adult animals (i.e., DMBA-induced mammary tumors), reflected in the ASF significantly exceeding unity, while for juvenile males there is no significant difference with adults and the ASF is consistent with unity.

Figure 9. Juvenile ASF Distributions



♦ Adult comparison group dosed on days 80-87
+ Adult comparison group dosed on days 140-147
* Adult comparison group dosing began during late juvenile window (day 46) and continued through day 61

Juvenile ASF Mixture Distributions

Since only one chemical, DMBA, had more than one study and the ASF differences for this chemical were moderate, the three methods used to generate a juvenile ASF mixture distribution

produced similar results. Figure 10 displays the juvenile ASF mixture cumulative distribution functions determined via Methods 1 through 3. The mean, and certain percentiles for each method are provided in Table 8. The juvenile ASF mixture cumulative distribution derived via Method 1 is nearly indistinguishable from the ASF mixture cumulative distribution derived via Method 2. The comparative length of the boxplots and their associated 90% confidence intervals between the DMBA exposed male and female rat bioassay studies (shown in Figure 9) are similar such that the inverse-variance weighting method produces a nearly identical ASF mixture distribution in comparison to Method 1. Method 3 results in greater differences in the ASF mixture distribution as compared to Methods 1 and 2 because the female DMBA rat ASF distribution is solely being sampled to represent the chemical DMBA. The female DMBA rat ASF distribution consists of ASF values that are entirely above unity. As a result, the ASF mixture cumulative distribution function via Method 3 is shifted to the right as compared to Methods 1 and 2. The difference observed is reflective of the greater sensitivity of female rats to mammary (i.e., breast) cancer during the juvenile period.

Figure 10. Juvenile ASF Mixture Cumulative Distribution Functions

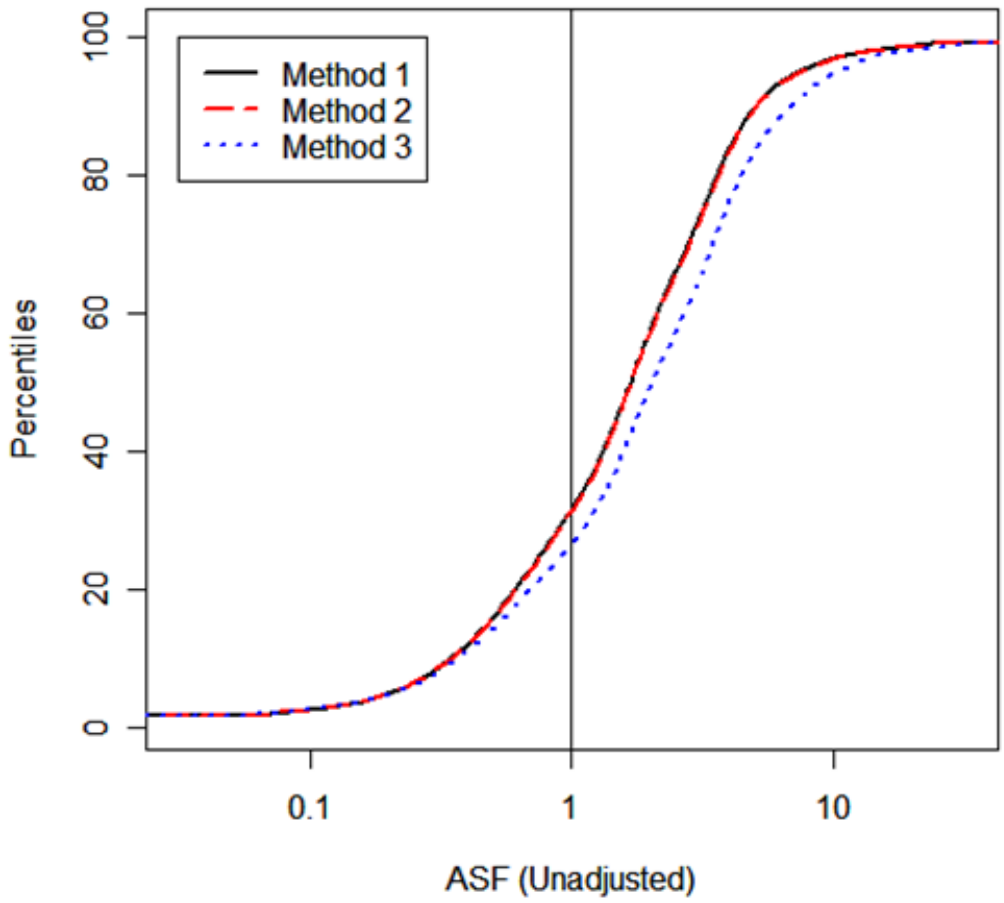


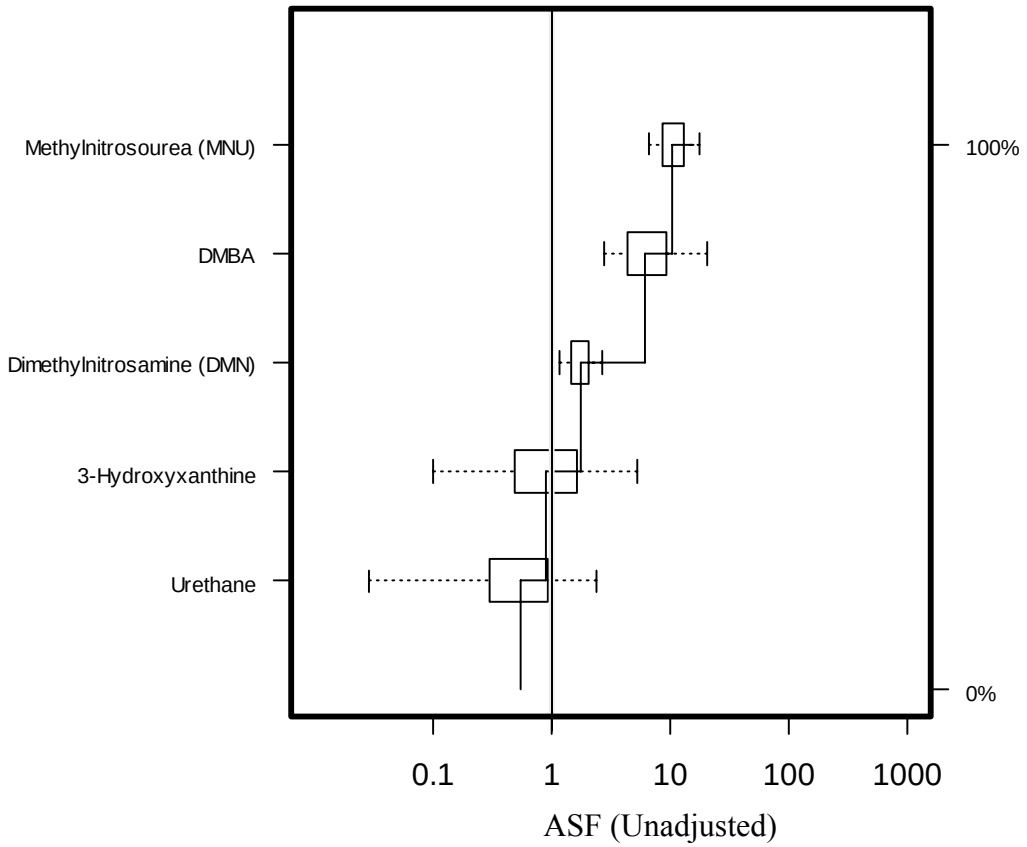
Table 8. Juvenile ASF Mixture Distribution Statistics by Method

Statistics	Unadjusted			Adjusted		
	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3
Mean*	2.63	2.71	3.49	7.10	7.32	9.42
Percentiles						
5th	0.20	0.20	0.20	0.54	0.54	0.54
10th	0.34	0.34	0.36	0.92	0.92	0.97
20th	0.60	0.61	0.69	1.62	1.65	1.86
30th	0.93	0.94	1.16	2.51	2.54	3.13
40th	1.31	1.33	1.58	3.54	3.59	4.27
50th	1.67	1.68	2.03	4.51	4.54	5.48
60th	2.10	2.13	2.69	5.67	5.75	7.26
70th	2.77	2.80	3.44	7.48	7.56	9.29
80th	3.57	3.62	4.43	9.64	9.77	11.96
90th	4.96	5.04	6.74	13.39	13.61	18.20
95th	7.29	7.46	10.16	19.68	20.14	27.43

* Calculated excluding large values above the 99th percentile.

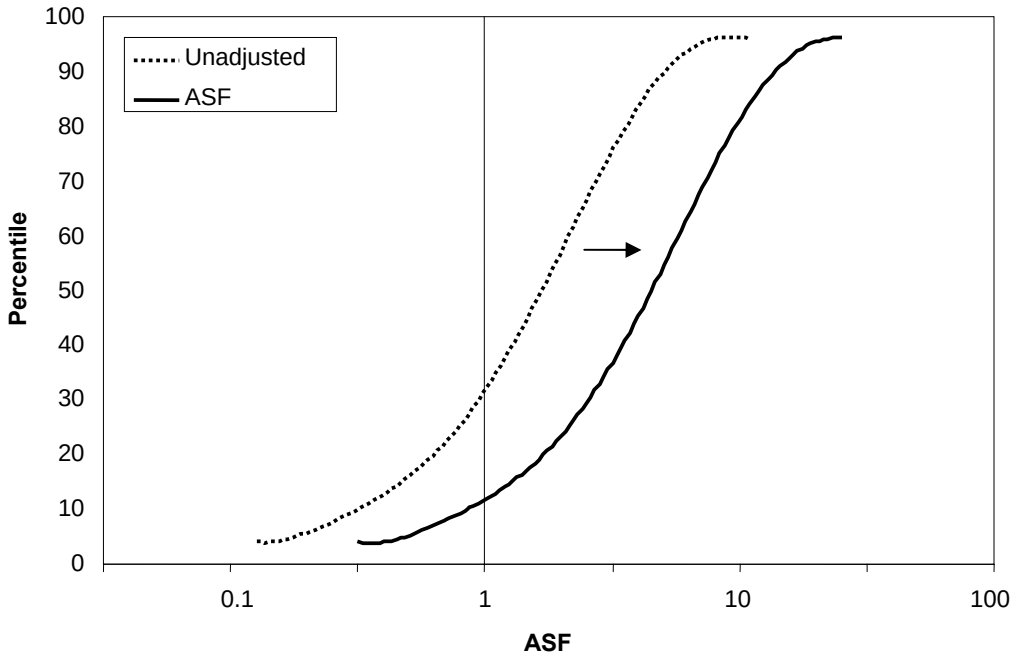
Figure 11 presents the boxplots for individual ASFs used to generate the Method 3 juvenile ASF mixture frequency distribution.

Figure 11. Juvenile ASF Cumulative Frequency Profile



As indicated in Figure 12 by the ASF curve, once an adjustment is made for timing of exposure, the difference between possible contributions of juvenile versus adult exposures becomes more pronounced for the cases examined here.

Figure 12. Method 1 Juvenile ASF Mixture Distribution



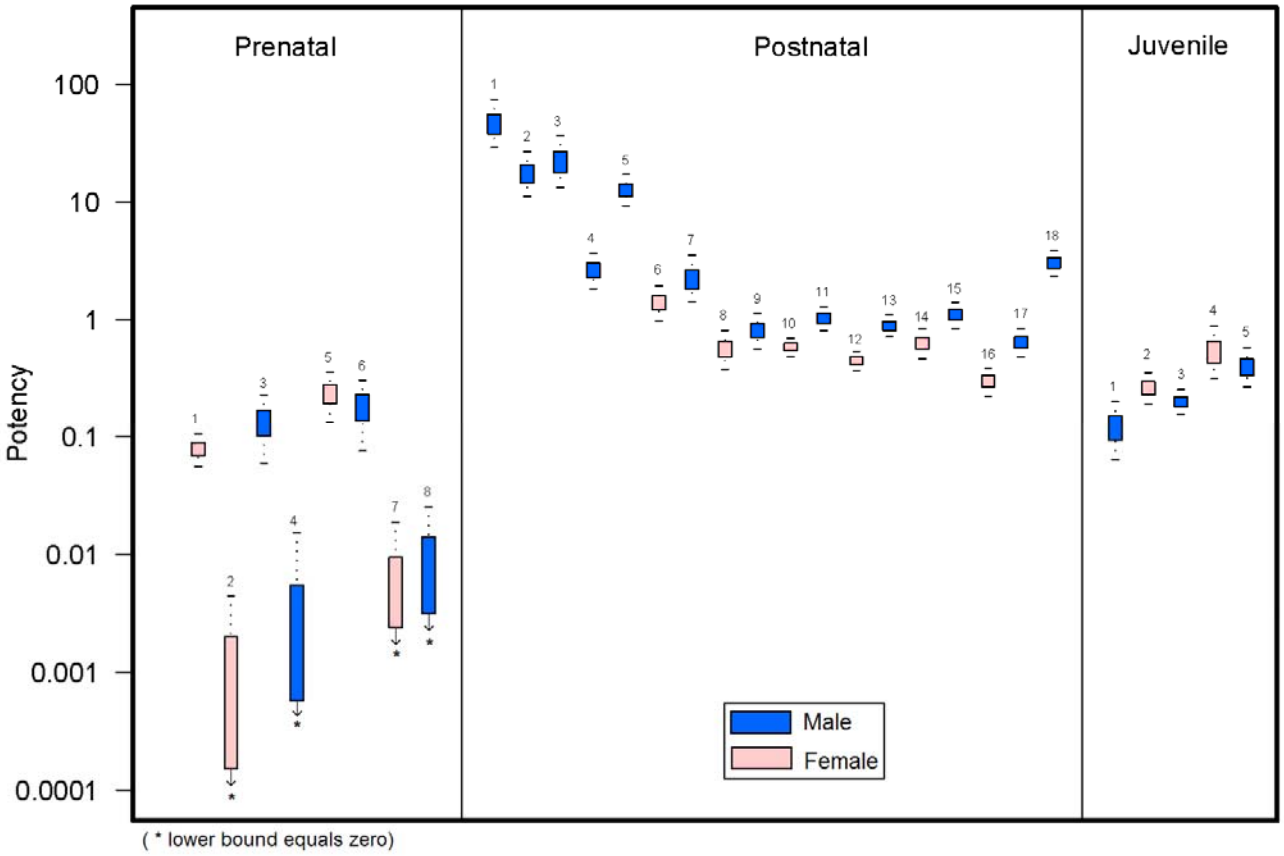
DEN Case Study

Ten mouse publications on DEN were included in the compilation of studies with early life exposure (See Table 5). Of these, three included groups of mice exposed during the prenatal window, seven included groups of mice exposed during the postnatal window, and two included groups of mice exposed during the juvenile window. These studies yielded a total of eight prenatal datasets, 18 postnatal datasets, and five juvenile datasets. No "adult only" exposure studies were identified in mice for DEN. Thus the juvenile exposure studies were used as the "later life" exposure comparison group. As noted earlier, if mice exposed to DEN during the juvenile lifestage are more prone to cancer than fully mature animals exposed to DEN, then the use of these juvenile exposure studies as the comparison group will result in an overall underestimate of the comparative cancer susceptibility of exposures during the prenatal and postnatal windows.

Cancer Potency Distributions

Figure 13 displays the box plots representing the cancer potencies derived for the different DEN prenatal, postnatal and juvenile exposure window studies in the mouse. The interquartile range of the potency distributions is shown as boxes, while the upper and lower bars extend from the box to the 95^{th} and 5^{th} percentiles, respectively. The Appendix D tables give the numerical values for these bounds, along with the mean, standard deviation, and median for each of the displayed distributions. The prenatal potency distributions fall into two distinct groupings. One grouping is located about the potency value 0.1. The second grouping is centered approximately at the potency value 0.005. The second grouping of studies exhibits greater fold-variability than the first grouping. The postnatal potency distributions all have confidence intervals that are entirely above the potency value of 0.1. Graphically, a greater cancer risk for mice exposed during the postnatal exposure window as compared to the prenatal window is apparent. The juvenile potency distributions also have slightly elevated potency values compared to those derived from the prenatal studies.

Figure 13. Cancer Potencies for DEN in Mice Exposed in Prenatal, Postnatal or Juvenile Exposure Windows



Prenatal Exposure

1 Anderson *et al.* (1989), C3H/HeN, F, sac day 540
2 Ibid, sac day 650
3 Ibid, M, sac day 461
4 Ibid, sac day 644
5 Mohr and Althoff (1965), NMRI, F
6 Ibid, M
7 Vesselinovitch (1983), B6C3F1, F
8 Ibid, M

Postnatal Exposure

1 Boberg *et al.* (1983), B6C3F1, M
2 Drinkwater and Ginsler (1986), B6C3F1, M
3 Ibid, C3H/HeJ, M
4 Ibid, C57BL/6J, M
5 Lai *et al.* (1985), B6C3F2, M
6 Rao and Vesselinovitch (1973), B6C3F1, F
7 Ibid, M
8 Turusov *et al.* (1973), CF-1, F
9 Ibid, M
10 Vesselinovitch *et al.* (1984), B6C3F1, F, PND 1
11 Ibid, M, PND 1
12 Ibid, F, PND 15
13 Ibid, M PND 15
14 Ibid, C3AF1, F, PND 1
15 Ibid, M, PND 1
16 Ibid, F PND 15
17 Ibid, M PND 15
18 Vesselinovitch. (1980), B6C3F1, M

Juvenile Exposure

1 Rao and Vesselinovich (1973), B6C3F1, M
2 Vesselinovitch *et al.* (1984), B6C3F1, F
3 Ibid, M
4 Ibid, C3AF1, F
5 Ibid, M

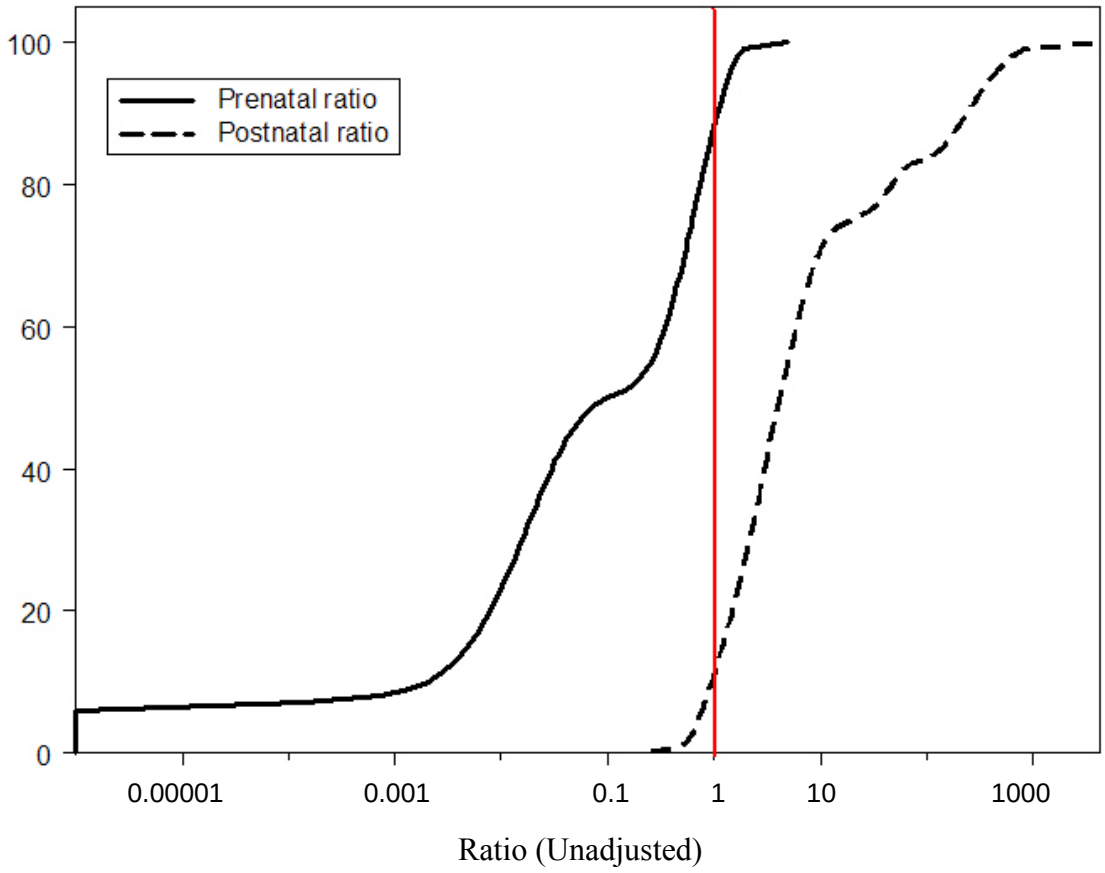
"Early Life" vs. "Later Life" Exposure Ratio Distributions

For each of the methods described in the Methods section above, mixture potency distributions were calculated for the prenatal, postnatal and juvenile exposure windows. These distributions were used then to calculate ratios of prenatal to juvenile potencies and postnatal to juvenile potencies. The output of the analysis is also a distribution. These are referred to as the DEN prenatal ratio distribution and the DEN postnatal ratio distribution. The term ASF is not used because they do not represent differences from early vs. adult exposures, but rather prenatal and postnatal vs. juvenile exposure.

Method 1: Equal Weighting of Potency Distributions within an Exposure Window.

Figure 14 shows the DEN prenatal and postnatal ratio cumulative distribution functions generated using Method 1. The differences in sensitivity to DEN among the prenatal and postnatal exposure windows are evident, with animals exposed *in utero* exhibiting considerably less sensitivity than those exposed postnatally.

Figure 14. Method 1 DEN Prenatal and Postnatal Ratio Cumulative Distribution Functions – Equal Weighting of Potency Distributions



The percentiles for the prenatal and postnatal ratio distributions are provided in Table 9. The 88th percentile of the prenatal ratio distribution is slightly less than unity. The distributional statistics indicate that mice exposed during the prenatal age window are less prone to the tumorigenic effects of DEN as compared to those exposed as juveniles. In contrast, the 11th percentile of the postnatal ratio distribution is greater than unity, thus 89% of the distribution indicates that mice exposed during the postnatal age window are more prone to the tumorigenic effects of DEN than those exposed as juveniles. The distributional differences in cancer risk (as compared to juveniles) between DEN exposures occurring during a prenatal window versus a postnatal window are quite evident.

In the variation on Method 1, where the potency distributions derived from each experiment are truncated at the 5th and 95th percentiles, the results are not appreciably different from those obtained without the truncation, and indicate the same general conclusions.

Table 9. Method 1 DEN Prenatal and Postnatal Ratio Distributions (Unadjusted) – Equal Weighting of Potency Distributions

Percentiles	Method 1		Method 1 (truncated)	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	0.00	0.74	0.00	0.76
10th	0.002	0.96	0.002	0.98
20th	0.008	1.50	0.007	1.51
30th	0.02	2.19	0.01	2.20
40th	0.03	3.00	0.03	2.98
50th	0.10	4.21	0.10	4.21
60th	0.35	6.01	0.36	5.99
70th	0.53	9.53	0.53	9.31
80th	0.75	47.51	0.74	46.84
90th	1.08	240.62	1.06	239.10
95th	1.36	408.95	1.30	393.52

Figure 15 shows the DEN prenatal and postnatal ratio frequency distributions generated using Method 1. Both the prenatal and postnatal ratio frequency distributions are multi-modal.

Figure 15. Method 1 DEN Prenatal and Postnatal Ratio Frequency Distributions – Equal Weighting of Potency Distributions

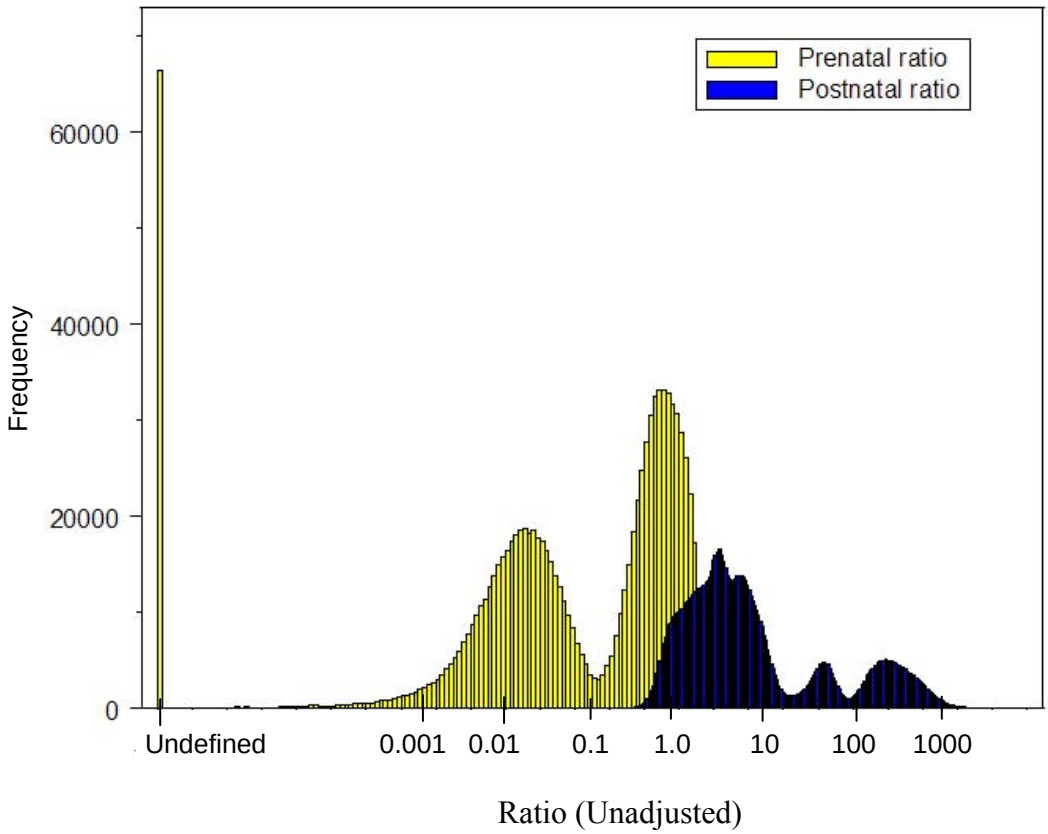


Table 10 shows the ratios when an adjustment is made for early versus adult timing of exposure. In this case, at approximately the 60th percentile, the prenatal ratio indicates equal contribution to lifetime risk from adult and *in utero* exposure. The postnatal ratio indicates considerably greater contributions to risk from exposures early in the postnatal period.

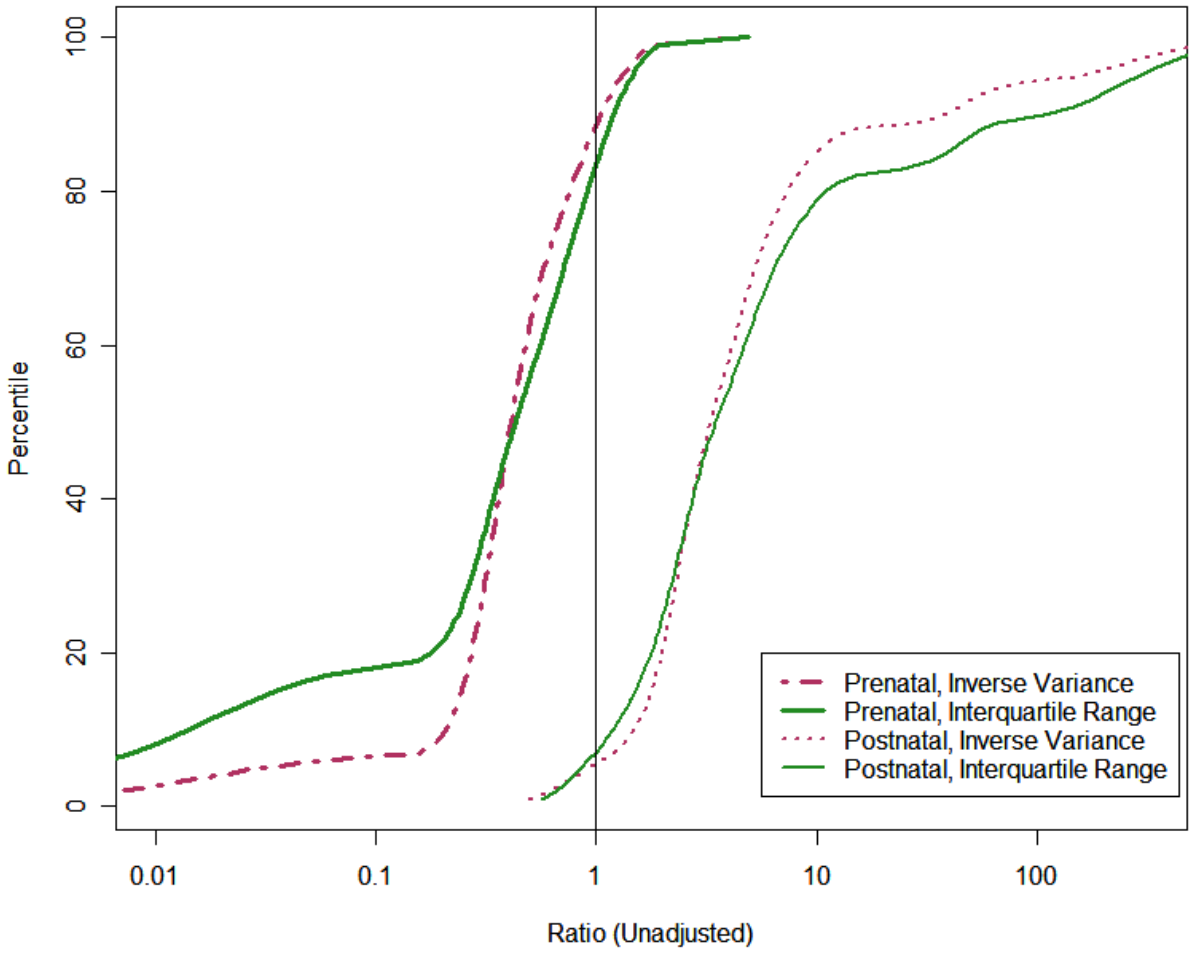
Table 10. Method 1 DEN Prenatal and Postnatal Ratio Distributions – with Adult vs. Early-Life Timing of Exposure Adjustment

Percentiles	Prenatal Ratio	Postnatal Ratio
5th	0.00	2.14
10th	0.01	2.78
20th	0.02	4.34
30th	0.05	6.37
40th	0.09	8.70
50th	0.31	12.20
60th	1.05	17.43
70th	1.58	27.65
80th	2.24	137.79
90th	3.25	697.81
95th	4.07	1185.95

Method 2: Weighting Potency Distributions by Inverse-Variance and the Interquartile Range.

Figure 16 shows the DEN prenatal and postnatal ratio cumulative distribution functions generated using Method 2a, weighting by inverse-variance, and Method 2b, weighting by the interquartile range (IQR). Qualitatively the results are similar to Method 1, with considerable sensitivity exhibited in the postnatal window. The magnitude of the differences in the ratio distributions for DEN across the prenatal and postnatal exposure windows is evident.

Figure 16. Methods 2a and 2b DEN Prenatal and Postnatal Ratio Cumulative Distribution Functions – Inverse-Variance and Interquartile Weighting of Potency Distributions



The percentiles for the prenatal and postnatal ratio distributions are provided in Table 11a (unadjusted) and b (adjusted for early versus adult timing of exposure). With inverse-variance weighting, slightly less than 89% of the unadjusted prenatal ratio distribution lies below the value of one. Although not statistically significant, the distributional statistics suggest that mice exposed during the prenatal age window are less prone to the tumorigenic effects of DEN as compared to those exposed as juveniles. For the unadjusted postnatal ratio distribution, more than 94% of the unadjusted postnatal ratio distribution is greater than unity under Method 2a (inverse-variance weighting), indicating that mice exposed during the postnatal age window are more prone to the tumorigenic effects of DEN than those exposed as juveniles. The

distributional differences in cancer risk (as compared to juveniles) between DEN exposures occurring during a prenatal window versus a postnatal window are quite evident.

Table 11a. Method 2 DEN Prenatal and Postnatal Ratio Distributions (Unadjusted)– Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	0.03	0.93	0.005	0.85
10th	0.21	1.49	0.01	1.19
20th	0.27	1.99	0.18	1.77
30th	0.32	2.34	0.27	2.24
40th	0.36	2.74	0.34	2.73
50th	0.41	3.31	0.43	3.48
60th	0.47	4.18	0.55	4.71
70th	0.58	5.27	0.71	6.42
80th	0.75	7.36	0.91	11.02
90th	1.04	37.80	1.20	106.70
95th	1.30	154.34	1.45	287.68

Table 11b. Method 2 DEN Prenatal and Postnatal Ratio Distributions (Adjusted*)– Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	0.09	2.69	0.02	2.47
10th	0.62	4.33	0.03	3.45
20th	0.81	5.78	0.54	5.13
30th	0.95	6.80	0.81	6.50
40th	1.08	7.94	1.02	7.92
50th	1.23	9.60	1.29	10.09
60th	1.42	12.12	1.65	13.66
70th	1.73	15.27	2.13	18.62
80th	2.25	21.35	2.73	31.96
90th	3.13	109.62	3.60	309.43
95th	3.90	447.59	4.35	834.27

*Adult vs. early-life timing of exposure adjustment

ENU Case Study

Thirteen mouse publications on ENU were included in the compilation of studies with early life exposure (See Table 5). Of these, five included groups exposed during the prenatal window, eight included groups exposed during the postnatal window, and three included groups exposed during the juvenile window. These studies yielded a total of 30 prenatal, 27 postnatal, and eight juvenile experiments. As with DEN, no "adult only" exposure studies were available and the juvenile exposure studies were used as the "later life" exposure comparison group.

Cancer Potency Distributions

Figure 17 displays boxplots representing the cancer potencies derived from the ENU mouse experiments. The interquartile range of the potency distributions is shown as boxes, while the upper and lower bars extend from the box to the 95^{th} and 5^{th} percentiles, respectively. The Appendix E tables give the numerical values for these bounds, along with the mean, standard deviation, and median for each of the displayed distributions. The prenatal potency distributions fall into two distinct groupings. One grouping is located about the potency value 4.0, and a second grouping is centered approximately at the potency value 0.1. The grouping of prenatal studies with potency values centered around 4.0 have greater variability than the prenatal studies centered around the lower potency value of 0.1.. The postnatal potency distributions also exhibit two distinct groupings, with one grouping located about the potency value 0.7, and a second centered approximately at the potency value 0.1. The grouping of postnatal studies centered around 0.7 have greater variability than the postnatal studies centered around the lower potency value of 0.1. Finally, two distinct groupings are also apparent for the juvenile exposure window studies. One grouping is located about the potency value 0.05. The second grouping is centered approximately at the potency value 0.007. The grouping of juvenile studies centered about the potency value of 0.007 has greater variability than the grouping of juvenile studies centered about the higher potency value of 0.05.

Figure 17. Cancer Potencies for ENU in Mice Exposed in Prenatal, Postnatal or Juvenile Exposure Windows

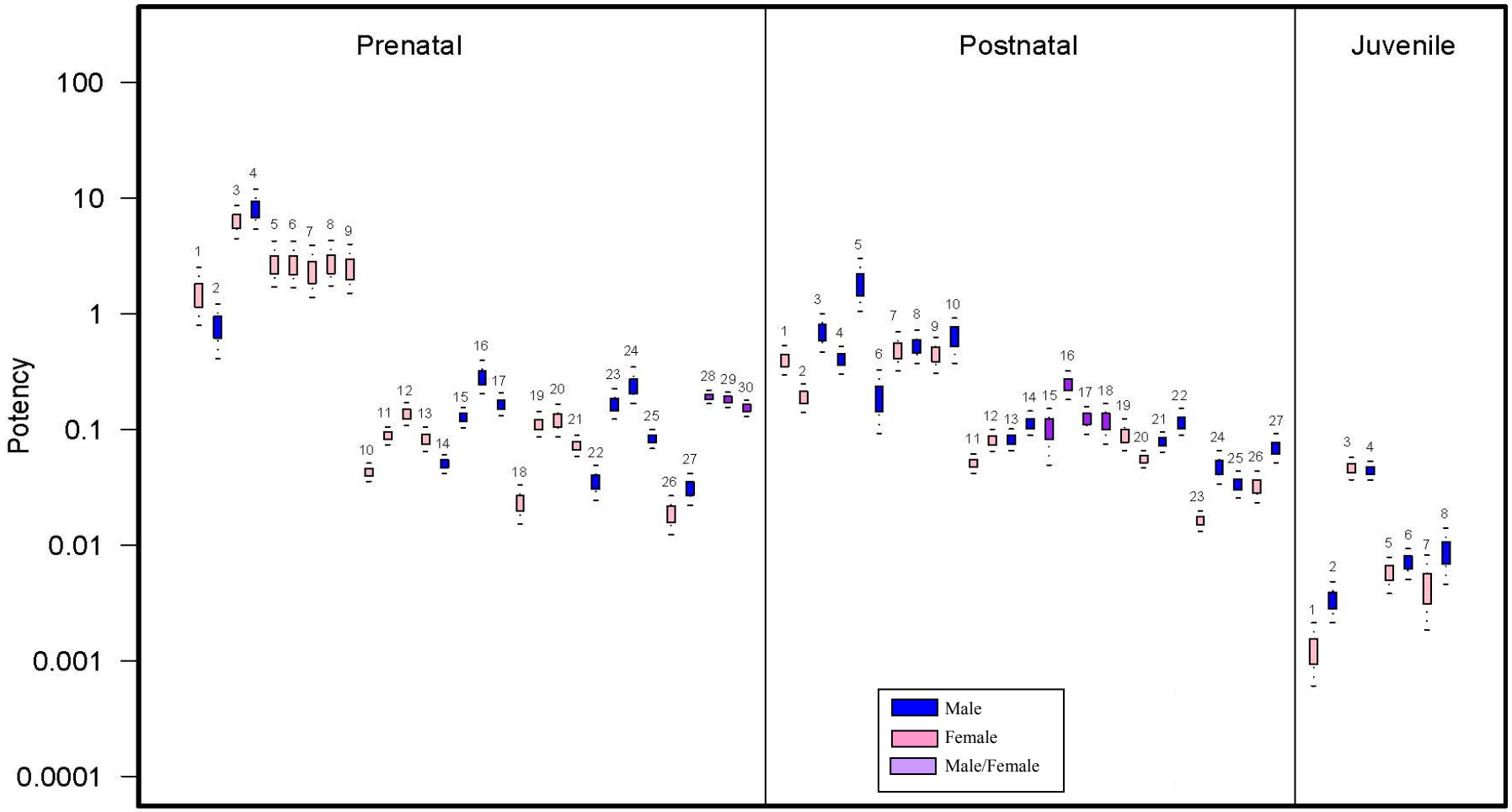


Figure 17 continued: Figure Legend

Prenatal Exposure

1 Diwan *et al.* (1974), AKR/J x SW/J, M
2 Ibid, AKR/J x SW/J , F
3 Ibid, SW/J x AKR/J , M
4 Ibid, SW/J x AKR/J, F
5 Kauffman (1976), Swiss, F
6 Ibid, Swiss, F sac day -6
7 Ibid, Swiss, F sac day -5
8 Ibid, Swiss, F sac day -4
9 Ibid, Swiss, F sac day -3
10 Vesselinovitch *et al.* (1977), sac -10
11 Ibid, B6C3F1, sac day -8
12 Ibid, B6C3F1, sac day -6
13 Ibid, B6C3F1, sac day -4
14 Ibid, B6C3F1, sac day -10
15 Ibid, , B6C3F1 Sac day 8
16 Ibid, B6C3F1 , Sac day 6
17 Ibid, B6C3F1, Sac day 4
18 Ibid, C3B6F1, Sac day 10
19 Ibid, C3B6F1, Sac day 8
20 Ibid, C3B6F1, Sac day 6
21 Ibid, C3B6F1, Sac day 4
22 Ibid, C3B6F1, Sac day 10
23 Ibid, C3B6F1, Sac day 8
24 Ibid, C3B6F1, Sac day 6
25 Ibid, C3B6F1, Sac day 4
26 Vesselinovitch (1983), B6C3F1, F
27 Ibid, B6C3F1, M
28 Wiggenhauser and Schmahl (1987), NMRI, sac day ,8
29 Ibid, NMRI, sac day ,7
30 Ibid, NMRI, sac day ,-6

Postnatal Exposure

1 Anderson *et al.* (1989), C3H/HenCr MTV,F, sac day 405
2 Ibid, C3H/HenCr MTV,F, sac day 451
3 Ibid, C3H/HenCr MTV,M , sac day 342
4 Ibid, C3H/HenCr MTV,M, sac day, 397
5 Drinkwater and Ginsler (1986), C3F/HeJ, M
6 Ibid, C57BL/6, M
7 Naito *et al.* (1982), A/He, F
8 Ibid, A/He, M
9 Pereira *et al.* (1985), Cd1, F
10 Ibid, CD1, M
11 Schmahl (1988), NMRI, F
12 Ibid, NMRI, F (independent exp)
13 Ibid, NMRI, M
14 Ibid, NMRI, M (independent exp)
15 Searle and Jones (1976), A, M/F,,
16 Ibid, C57BL, M/F
17 Ibid, DBA, M/F
18 Ibid, IF, M/F
19 Vesselinovitch *et al.* (1974), B6C3F1, F, day 1
20 Ibid, B6C3F1, F, day 15
21 Ibid, B6C3F1, M,day 1
22 Ibid, B6C3F1, M, day 15
23 Ibid, C3AF1,F
24 Ibid, C3AF1,M, day 1
25 Ibid, C3AF1,M, 15
26 Vesselinovitch (1983), B6C3F1,F
27 Ibid, B6C3F1, M

Juvenile Exposure

1 Vesselinovitch *et al.* (1973), B6C3F1, F
2 Ibid, B6C3F1, M
3 Vesselinovitch *et al.* (1974),C3AF11
4 Ibid, C3AF1, M
5 Ibid, B6C3F1, F
6 Ibid, B6C3F1, M
7 Vesselinovitch (1983), B6C3F1, F
8 Ibid, B6C3F1, M

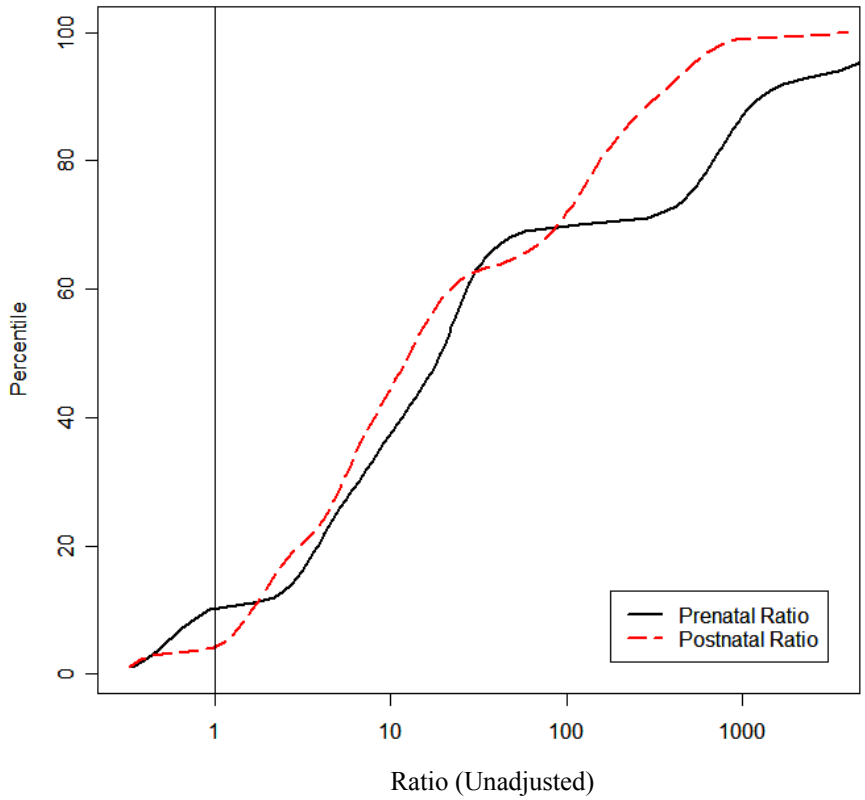
"Early Life" vs. "Later Life" Exposure Ratio Distributions

Using the same methods as described for DEN, ratios of the prenatal to the juvenile mixture potency distributions, and postnatal to juvenile mixture potency distributions were computed.

Method 1: Equal Weighting of Potency Distributions within an Exposure Window.

Figure 18 shows the ENU prenatal and postnatal ratio cumulative distribution functions generated using Method 1. In contrast to DEN, the sensitivity of mice to ENU in both the prenatal and postnatal windows is evident.

Figure 18. Method 1 ENU Prenatal and Postnatal Ratio Cumulative Distribution Functions – Equal Weighting of Potency Distributions



The percentiles for the prenatal and postnatal ratio distributions are provided in Table 12. Almost ninety percent of the prenatal ratio distribution exceeds unity, twenty-eight percent is between unity and 10, and sixty-two percent is greater than 10. The largest mode in the prenatal distribution is a ratio greater than 4000 (Figure 19). These observations indicate that mice

exposed during the prenatal age window are more prone to the tumorigenic effects of ENU than those exposed as juveniles.

More than 95% of the postnatal ratio distribution is greater than unity indicating that mice exposed during the postnatal age window are more prone to the tumorigenic effects of ENU than those exposed as juveniles.

Table 12. Method 1 ENU Prenatal and Postnatal Ratio Distributions (Unadjusted) – Equal Weighting of Potency Distributions

Percentiles	Method 1		Method 1 (truncated)	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	0.53	1.14	0.53	1.18
10th	0.94	1.65	0.93	1.68
20th	3.86	3.03	3.89	3.00
30th	6.56	5.39	6.59	5.45
40th	11.60	8.09	11.63	8.07
50th	19.30	12.84	19.40	12.81
60th	27.13	21.87	26.66	20.76
70th	116.16	88.96	137.82	92.27
80th	679.56	154.90	687.33	152.78
90th	1266.12	325.80	1173.53	319.53
95th	4381.63	519.75	4557.69	506.81

Figure 19 shows the ENU prenatal and postnatal ratio frequency distributions generated using Method 1. Both the prenatal and postnatal ratio frequency distributions are multi-modal. The ENU postnatal ratio distribution has a similar overall shape as the prenatal ratio distribution, with a shift to the left such that the values of the distribution are not as extreme. The ENU postnatal ratio distribution is more compact and lacks the most extreme values observed in the rightmost tail of the prenatal ratio distribution (Figure 18), although large values in the upper tails are evident (See also Table 12). Table 12 shows the ENU ratios calculated using Method 1 barely differ when the potency distributions are truncated at the fifth and ninety-fifth percentiles to eliminate the extreme values, prior to developing the mixture potency distributions.

Figure 19. Method 1 ENU Prenatal and Postnatal Ratio Frequency Distributions – Equal Weighting of Potency Distributions

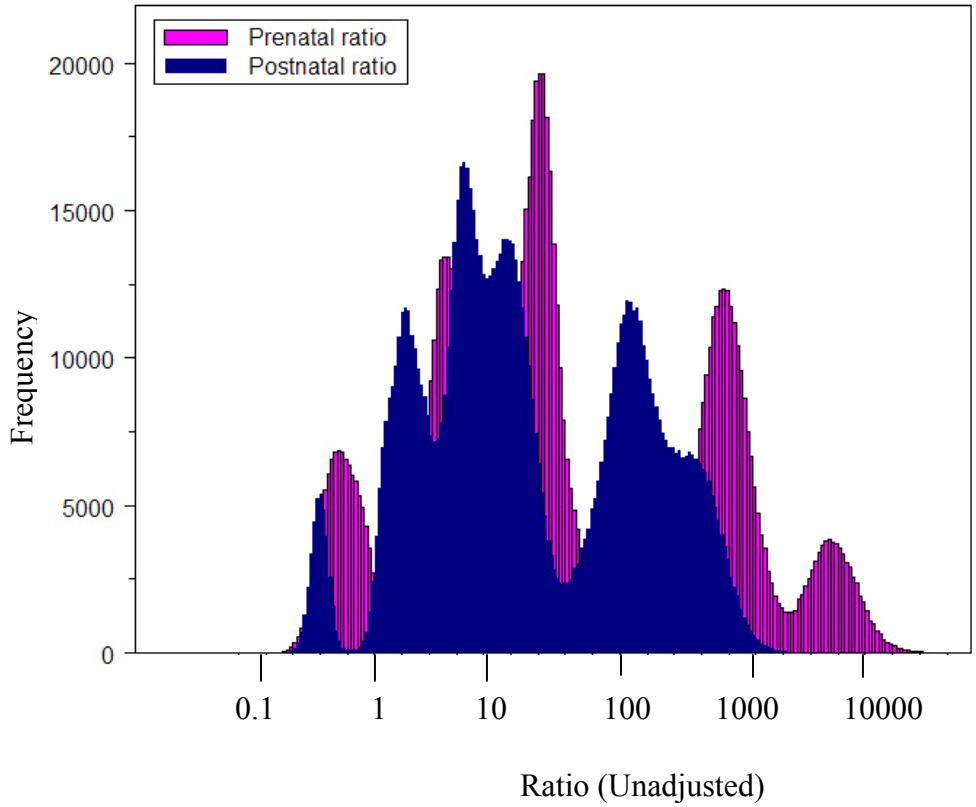


Table 13 shows the ratios calculated using Method 1, after adjustment is made for early versus adult timing of exposure.

Table 13. Method 1 ENU Prenatal and Postnatal Ratio Distributions – with Adult vs. Early-Life Timing of Exposure Adjustment

Percentiles	Prenatal Ratio	Postnatal Ratio
5th	1.59	3.31
10th	2.82	4.78
20th	11.58	8.79
30th	19.68	15.63
40th	34.80	23.46
50th	57.90	37.24
60th	81.39	63.42
70th	348.48	257.98
80th	2038.68	449.21
90th	3798.36	944.82
95th	13144.89	1507.28

Method 2: Weighting Potency Distributions by Inverse-Variance and Interquartile Range. The ENU prenatal and postnatal ratio distributions computed by Method 2a and Method 2b differ substantially from one another, as shown in Figure 20. This is because each exposure window has a grouping of experiments that have narrower confidence intervals than the remaining grouping of experiments. Within each exposure window, those experiments with the narrowest confidence intervals are given greater weight. Figure 20 demonstrates that the differences observed between the weighting methods is due to greater weight being assigned to these studies with the narrowest confidence intervals via the inverse-variance weighting method compared to the interquartile range weighting method.

The ENU prenatal ratio distributions computed via Method 2a and 2b have medians equal to 3.81 and 11.05, respectively. The ENU postnatal ratio distributions computed via Method 2a and 2b have medians equal to 0.55 and 7.24, respectively. Clearly, the inverse-variance weighting results suggest less susceptibility from early life exposure to ENU than the interquartile range weighting results. The inverse-variance weighting scheme tends to weigh the studies with narrower distributions, and in the case of the ENU pre- and postnatal studies, smaller potency values, considerably more heavily as compared to interquartile range weighting.

Both weighting methods clearly indicate greater inherent sensitivity of the prenatal window to ENU, which was also observed when studies were weighted equally (Method 1). The two weighting methods (2a and 2b) yield strikingly different results for the postnatal window, however. Using inverse variance weighting, approximately half of the ENU postnatal ratio distribution is less than unity, indicating no substantial inherent sensitivity for the postnatal compared to juvenile development window. With interquartile weighting, the 10^{th} percentile is 1.04 and half the distribution exceeds 7.0, indicating a strong postnatal sensitivity. The inverse variance results are also substantially different to the results seen when all studies are equally sampled, as shown in Method 1 above. However, the interquartile range weighting results are similar to those obtained via Method 1 though slightly more moderate. Results from both Method 2a and 2b indicate that prenatal sensitivity is substantially greater than postnatal sensitivity.

Figure 20. Methods 2a and 2b ENU Prenatal and Postnatal Ratio Cumulative Distribution Functions – Inverse-Variance and Interquartile Weighting of Potency Distributions

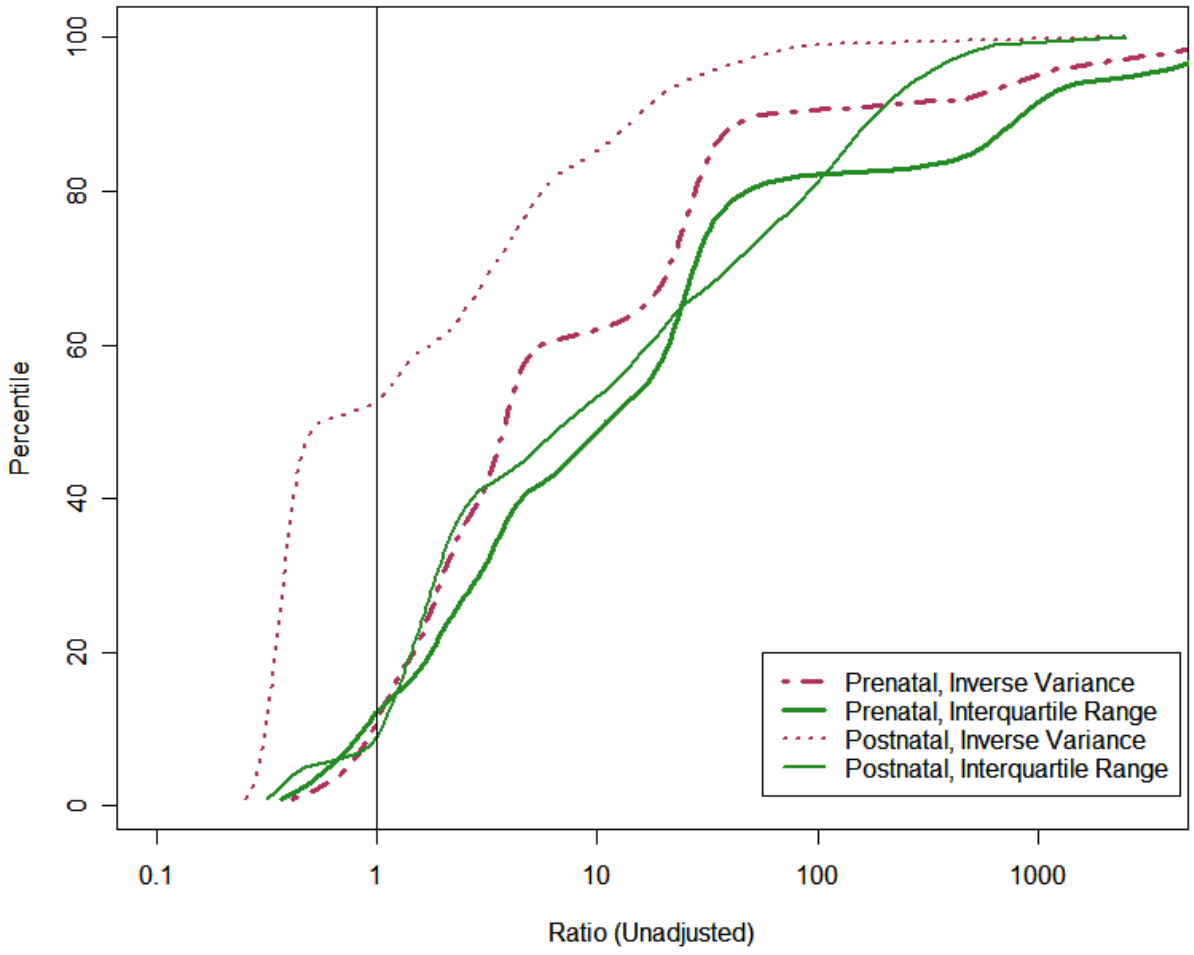


Table 14a. Method 2 ENU Prenatal and Postnatal Ratio Distributions (Unadjusted)– Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	0.74	0.29	0.61	0.47
10th	0.95	0.31	0.87	1.04
20th	1.45	0.35	1.75	1.43
30th	1.98	0.38	2.91	1.85
40th	2.93	0.42	4.55	2.69
50th	3.81	0.55	11.05	7.24
60th	5.45	1.72	20.97	17.05
70th	21.18	3.33	27.36	39.81
80th	27.75	5.61	47.64	91.56
90th	53.70	15.32	852.11	182.93
95th	940.28	27.92	2608.68	296.87

Table 14b. Method 2 ENU Prenatal and Postnatal Ratio Distributions (Adjusted*) – Distributional Weighting of Potency Distributions

Percentiles	Method 2a – Inverse Variance Weighting		Method 2b - Interquartile Weighting	
	Prenatal Ratio	Postnatal Ratio	Prenatal Ratio	Postnatal Ratio
5th	2.22	0.78	1.83	1.36
10th	2.85	0.84	2.61	3.02
20th	4.35	0.94	5.25	4.15
30th	5.94	1.03	8.73	5.37
40th	8.79	1.13	13.65	7.80
50th	11.43	1.48	33.15	21.00
60th	16.35	4.64	62.91	49.45
70th	63.54	8.99	82.08	115.45
80th	83.25	15.15	142.92	265.52
90th	161.1	41.36	2556.33	530.50
95th	2820.84	75.38	7826.04	860.92

*Adult vs. early-life timing of exposure adjustment

Discussion

Data from studies on 23 unique carcinogens, 20 of which are considered to act via primarily genotoxic modes of action, were analyzed. Of these 20 carcinogens, 16 are thought to require metabolic activation to the ultimate carcinogenic species. The analyses indicate that both the prenatal and postnatal lifestages can be much more susceptible to developing cancer than the adult lifestage. As an index of inherent susceptibility, one that does not account for the longer time early exposures can manifest, an ASF (unadjusted) was derived. This index compares the carcinogenicity activity when exposures occur early in life compared to older ages, for the same period of time between initial exposure and observation of effect. For the multi-window studies, the median unadjusted ASF for the postnatal period was 4.6 or 7.5, and the upper 95% confidence bound ranged from 123 to 188, depending on the method of combining the ASF distributions underlying studies on the same chemical.

There were few cases of unadjusted ASFs less than 1 for the postnatal window. These results indicate that in general, for the chemicals studied, there is inherently greater susceptibility during the early postnatal compared to the adult period. The differences between postnatal and adult susceptibility appear more pronounced once an adjustment is made to the ASF to take into account the longer period cancer has to manifest when exposure occurs early in life. The median value for the adjusted ASF indicates for the chemicals studied here either a 13.4- or 21.6- fold greater contribution to lifetime cancer risk when exposure occurs during this period, compared to the same exposure averaged throughout the adult period; the upper 90^{th} percentile ASF ranged from 211 to 307.6, depending on the method for combining the ASF distributions for the underlying studies. The DEN and ENU case studies also exhibited substantial sensitivity in the postnatal period, with inherent susceptibility about half an order of magnitude greater than juveniles for DEN, and about an order of magnitude greater than juveniles for ENU, and again larger contributions to risk during this period once the timing of exposure adjustment is made.

Regarding *in utero* exposure, few studies provided data indicative of equal inherent adult and prenatal susceptibility, with an unadjusted ASF of unity. For the multi-window studies, the

unadjusted ASF distribution is roughly bimodal, with unadjusted ASFs for several studies significantly greater than unity and several others significantly less than unity (Figure 1). The median unadjusted ASF ranges from 0.93 to 2.5, depending on the method used to combine studies on the same chemical. For ASFs adjusted to take into account the longer period for cancer to manifest for early life exposures, median estimates range from 2.8 to 7.5, and mean estimates from 16.6 to 37.1, depending on the method used to combine studies. This modality in the unadjusted ASF distribution for the prenatal window is reflected in the case studies. The prenatal vs. juvenile potency ratio for DEN has a median of 0.1 to 0.43, depending on the method used to combine studies within each exposure window, and the majority of the distribution falls below unity. This is suggestive of reduced inherent susceptibility *in utero*. In contrast the median unadjusted ASF for ENU range from 3.8 to 19.4, with the majority of the distribution exceeding unity, indicative of greater inherent *in utero* susceptibility. In considering implications of the DEN and ENU case studies it is important to recognize that the referent groups were juvenile rather than adult animals. The prenatal vs. juvenile and postnatal vs. juvenile ratios for these chemicals are likely to be underestimates of the ASF, to the extent that some of the apparent sensitivity for DEN and ENU in the early postnatal period carries through to the juvenile period.

ENU is a direct acting carcinogen that does not require metabolic activation to alkylate DNA, forming DNA adducts and mutations that ultimately result in the formation of tumors (Slikker III *et al.,* 2004). In contrast, DEN requires metabolic activation by cytochrome P450 enzymes (e.g., P450 2E1, P450 2A6) to form the active DNA ethylating species (Brittebo *et al.*, 1981). While both ENU and DEN cross the placenta and are widely distributed in fetal tissues (Rice *et al.* 1989; Brittebo *et al.*, 1981), DEN can not be metabolized to any significant extent by fetal tissues until relatively late in gestation (i.e., gestation day 18 in the mouse), and after birth the expression of P450 2E1 progressively increases, reaching adult levels by day 30 (Brittebo *et al.*, 1981). This may explain the lower fetal susceptibility of DEN. However, the multi-exposure window studies illustrate that *in utero* metabolic status is not the sole determinant of *in utero* susceptibility: benzidine and safrole require metabolic activation and exhibit greater susceptibility from *in utero* exposure (see Figure 1).

There are just five chemicals and seven studies, two of which are not independent (i.e., the MNU studies of Grubbs *et al*., 1983), available to examine susceptibility in the juvenile period. The unadjusted ASF indicates significantly greater susceptibility in this period for three of the independent studies, with the three remaining independent studies consistent with equal inherent susceptibility to adult animals (Figure 9). For the juvenile window, the ASFs adjusted for timing range from 4.5 to 5.5 at the 50^{th} percentile and from 19.7 to 27.4 at the 95^{th} percentile.

The studies that comprise the set of multi-window studies available for these analyses were not homogeneous. That is, they do not represent observations from the same distribution. Of the three methods used to combine the ASF distributions underlying studies within each exposure window, the method of equally weighting studies within a chemical appears to best represent the available data. The use of inverse variance in weighting ASF distributions within a chemical may underweight small studies and overweight large ones, and thus produce a mixture ASF distribution that does not accurately reflect the overall data. This is clearly illustrated by the results of the postnatal ENU case study analyses. The method of selecting a single study (i.e., that with the largest median ASF) to represent each chemical may also result in inadvertent bias if a selected study is not representative of the group being studied.

In adjusting the ASF to take into account the longer period of time for early carcinogen exposures to manifest, the hazard function was assumed to increase with the third power of age. If the true rate of increase with age is greater than that, then the ASFs presented here may result in underestimates of the true sensitivity of these early life stages.

As the multi-window and case studies show, there appears to be considerable variability in age-at-exposure related susceptibility across carcinogens. There is also variability in age-at-exposure related susceptibility among studies of the same carcinogen. The sources of variability evident in the analyzed studies include timing of exposure within a given age window, and gender, strain, and species differences in tumor response. The set of studies identified and analyzed was not sufficiently robust to fully describe quantitatively the variability. This variability raises concerns that selection of the median, that is the 50^{th} percentile, estimates for age window-specific ASFs may considerably underestimate effects for certain carcinogens or population

groups. Relatively large variability in humans in response to carcinogens is expected to be common (Finkel, 1995; 2002).

Several of the carcinogens studied induced tumors at multiple sites in the same experiment, and at different sites, depending upon the age window during which exposure occurred. The cancer potencies used in the early vs. later life comparisons were based on all treatment-related tumors. When treatment-related tumors were induced at multiple sites in the same experiment, or at the same site, but arising from different cell types, the slopes of the dose response curves from these different tumor sites or types were statistically combined to create an overall multisite cancer potency distribution for that experiment. The result reflects the total cancer impact associated with the carcinogen exposure in question. This approach differs from other researchers investigating early vs. late in life differences (e.g., Barton *et al.*, 2005; Hattis *et al.*, 2004; 2005). We believe this provides a more complete approach for considering age specific differences in carcinogenic activity.

One limitation of the approach was analysis of age windows, without attempting to describe changes in susceptibility within an age window. Timing of carcinogen exposure within a given age window can affect the cancer outcome observed. This is illustrated by experiments with 1-ethyl-1-nitrosobiuret in prenatal and adult rats by Druckrey and Landschutz (1971). A three fold difference in activity was observed between two prenatal exposure groups, one exposed on prenatal day -10 and the other on prenatal day -3 (See Figure 1 and Appendix B, Table B1). The timing of exposure within the adult age window can also affect the cancer outcome, as illustrated by the experiments of Grubbs *et al.* (1983), in which female rats exposed early in the adult period (days 80 through 87) were more than three times as sensitive to the breast cancer effects of MNU than females exposed six weeks later (Figure 9 and Appendix B, Table B3). In general the adult comparison groups in the multi-window studies were fairly young. The extent to which this may result in an overall bias of the results presented here is unclear. Also for several cases, juvenile animals were used as the later life exposure group. In these cases the ASFs are likely underestimates of the relative sensitivity of the prenatal and postnatal lifestages, compared to that of the adult lifestage.

Excluded from the analysis presented here were early in life studies in which exposure of a given exposure group crossed multiple age windows. An example of results from studies of this type is provided by mouse studies for two non-genotoxic carcinogens, diphenylhydantoin (Chhabra *et al.*, 1993a) and polybrominated biphenyls (Chhabra *et al.*, 1993ab), in which exposures began prior to conception, and continued throughout the prenatal, postnatal, and post-weaning periods, up to the age of eight weeks. The data, shown in Appendix F, demonstrate an increased sensitivity associated with exposures to either of these non-genotoxic carcinogens during the entire early life period, as compared to exposures during only the adult lifestage. Some studies that crossed multiple age windows were included in the analyses of Barton *et al.* (2005), which are consistent with the general conclusions here.

Barton *et al.* (2005) discussed data on 18 unique carcinogens, but ultimately analyzed data on six mutagenic carcinogens (benzidine, diethylnitrosamine, 3-MC, safrole, urethane, and vinyl chloride) to derive the age dependent adjustment factor of 10 for carcinogen exposures occurring between birth and the second birthday, as specified in the U.S. EPA's (U.S. EPA, 2005) *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens*. In performing the analysis, Barton *et al.* (2005) compared tumor site-specific potencies, while here multi-site cancer potency estimates provide the basis for comparison. Barton *et al.* (2005) also did not address prenatal or juvenile exposures in their analyses, nor was the issue of longer "shelf-life" addressed wherein exposure to the fetus, infant or child has a longer period of time compared to an exposed adult to produce cancer. Other evaluations of exposure occurring in early life and in adults in the same study have been attempted (e.g., McConnell, 1992) but have not considered indices of carcinogenic activity as systematically as was done in the analyses here or by Barton *et al.* (2005). Thus the analysis presented here adds to the body of evidence on which to consider methods to use in estimating cancer risk when the young are exposed.

Implications for Cancer Risk Assessment Guidelines

Taken together the results indicate that early lifestages are generally more sensitive to carcinogen exposure than adults, and that cancer risk assessment practices should take increased sensitivity of the young into account. Here the results of these analyses are reflected on in the context of existing state and federal cancer risk assessment guidelines. The degree that such guidelines adequately address carcinogenic exposures to the fetus, infants and children has been a concern

of the California State legislature, which mandated the study presented here, as part of the Children's Environmental Health Initiative (AB 2872, Shelly, HSC section 901). This legislation also required OEHHA to review its own and other Cal/EPA, state and federal guidelines to assess methodologies used and establish new methodologies if needed (HSC section 901 [b] and [c]).

U.S. EPA, California and other states now have legal mandates to ensure that regulatory standards are adequately protective of the fetus, infants and children, and have developed or are considering methodologies that explicitly address the young in cancer risk estimation. In California, the Children's Environmental Health Initiative (HSC section 901 [b]) mandates OEHHA to ensure that regulatory standards for carcinogens are adequately protective of fetuses, infants and children. In 2001 OEHHA reported on its review of existing guidelines. California has, on occasion, adjusted dose calculations used in estimating cancer potency with a Doll-Armitage analysis to account for variable dosing over time (e.g., early-in-life exposures). This model can be used to address the longer period of time available for cancer to manifest when exposures occur early in life. It does not however address the issue of inherent tissue susceptibility. OEHHA in 2001 concluded that the existing default mathematical models employed for the purpose of estimating excess cancer risk did not adequately address the possibility that risk from early-in-life exposures may differ from that associated with exposures occurring in adulthood. OEHHA further concluded that there was a need for such methodologies to be developed, tested, and validated (Cal/EPA, 2004). Also, under SB 25 (The Children's Environmental Health Protection Act of 1999, Escutia, HSC section 39600 *et seq*.), in re-evaluating cancer potency values under the Air Toxics Hot Spots program, California is required to take into account general or chemical-specific consideration which suggests that children may be especially susceptible to certain carcinogenic effects.

The U.S. EPA *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (U.S. EPA, 2005) concluded there is evidence of differential susceptibility for mutagenic carcinogens and recommended adjustments to the adult slope factor and its integration with exposure estimates in estimating cancer risk associated with early life exposures. A ten-fold adjustment to the adult slope factor is suggested for exposures to mutagenic carcinogens

occurring from birth up to two years of age, and a three-fold adjustment for such exposures occurring from 2 up to 16 years of age. No adjustment was recommended to address the fetus for increased susceptibility or the full lifetime ahead for cancer to be manifest. No adjustment was suggested for non-mutagenic carcinogens (U.S. EPA, 2005), even though there is increasing appreciation that carcinogens often act by multiple mechanisms, including non-mutagenic mechanisms, and that the relative importance of a given mechanism of action may vary with lifestage. Indeed, evidence from human cancers indicates that epigenetic changes, such as alterations in DNA methylation, are often associated with early events in human carcinogenesis (Baylin, 2005). Thus existing U.S. EPA guidance applies to only a subset of carcinogens, and, while addressing exposures to infants and children, does not acknowledge any effect of carcinogen exposures to the fetus.

OEHHA recognizes the limitations in the data and analyses presented, as discussed above. Still the analyses do provide some guidance on the extent risk may be over- or underestimated by current approaches. The analyses demonstrate the sensitivity of three early life exposure windows for the carcinogens analyzed here. While there is a great deal of variability across chemicals in the prenatal ASFs, the data indicate that the potency associated with prenatal carcinogen exposure is not zero. A factor of 10 falls roughly at the 70th percentile for the multi-window study analysis (Table 6). This value could be applied to the potency estimate when calculating lifetime cancer risk in humans arising from carcinogen exposures that occur *in utero*. Alternatively, factors of 50 and 115 fall roughly at the 90th and 95th percentiles, respectively, for the multi-window prenatal ASF analysis.

The U.S. EPA's factor of 10 for postnatal exposures falls between the 40th and 50th percentiles for postnatal studies (Table 7); thus while it is consistent with the data presented, it may result in underestimates of risk for a reasonable fraction of chemicals. Factors of 210 and 350 fall roughly at the 90th and 95th percentiles, respectively, for the multi-window postnatal ASF analysis. The U.S. EPA's factor of 3 for juvenile exposures is consistent with the range of estimates derived from the multi-window studies, although it falls below the median estimates for all three methods presented (Table 8). It is acknowledged that there are few data available on which to base an estimate for the juvenile period. A factor of 3 adjusts for the longer time it takes for cancer to manifest, but is unlikely to fully account for inherent differences in

susceptibility to cancer, such as occurs in breast tissue of pubescent girls exposed to radiation. Factors of 13 and 20 fall roughly at the 90th and 95th percentiles, respectively, for the multi-window juvenile ASF analysis.

Table 15 illustrates the impact of age-window specific ASFs on lifetime cancer risk. In this example, exposure to the carcinogen is assumed to occur at a constant exposure rate over the entire lifetime. Risk calculations were performed using the mean, 50th, 70th, and 95th percentile ASF values derived using Method 1 (i.e., equally weighting studies within a chemical; selected based upon the findings of a sensitivity analysis as the best method to represent the available data) to adjust the adult cancer potency. As shown in Table 15, when increased susceptibility of the fetus, infants, and children is taken into account by applying 50th percentile ASF values, the total lifetime cancer risk is increased two-fold; applying 70th percentile ASF values increases the risk three-fold, applying mean ASF values increases the risk 4.6-fold, and applying 95th percentile ASF values increases the risk 16-fold above the risk estimated in the absence of age-specific adjustments to the potency. Table 15 also shows how the application of the U.S. EPA's adjustment factors for the postnatal and juvenile age windows in calculating total lifetime cancer risk compares with the use of the ASF values derived from the multi-window studies analyzed here. For example, the use of 70th percentile ASF values as adjustments for the prenatal, postnatal, and juvenile age windows increases the total lifetime cancer risk almost two-fold above the risk estimated using the U.S. EPA's adjustment factors.

Concluding Remarks

This report indicates the extent risk may be over- or underestimated by current risk assessment approaches. The analyses support the application of weighting factors to address potential increased susceptibility to carcinogen exposures occurring prenatally and during postnatal and juvenile age periods. The limitations in the data and analyses are recognized and discussed in the report. Limitations can not explain the age specific differences observed.

Table 15. Comparison of cancer risk estimates[1] for lifetime exposure to 0.0001 mg/kg-d of a carcinogen with potency 1 (mg/kg-d)$^{-1}$ based on different parameters of ASF distributions[2], or U.S. EPA values.

Age window	Years of life exposed	No adjustment		50th percentile		70th percentile		Mean		95th percentile		U.S. EPA (2005)	
		ASF	Risk	ASF	Risk	ASF	Risk	ASF	Risk	ASF	Risk	Factor	Risk
In utero	0.75	0	0.0	3	3.2×10^{-6}	10	1.1×10^{-5}	21	2.2×10^{-5}	115	1.2×10^{-4}	0	0.0
Birth to <2 yr	2	1	2.9×10^{-6}	13	3.7×10^{-5}	28	7.9×10^{-5}	79	2.3×10^{-4}	350	1.0×10^{-3}	10	2.9×10^{-5}
2 to <16 yr	14	1	2×10^{-5}	5	1.0×10^{-4}	7	1.4×10^{-4}	7	1.4×10^{-4}	20	4.0×10^{-4}	3	6.0×10^{-5}
16 to 70 yr	55	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}	1	7.9×10^{-5}
Total lifetime risk			1.0×10^{-4}		2.2×10^{-4}		3.1×10^{-4}		4.7×10^{-4}		1.6×10^{-3}		1.7×10^{-4}

[1] Risk accrued in age window = potency x ASF x exposure rate x (years exposed/70 years).
[2] ASF derived using equal weighting of studies within a chemical (i.e., Method 1 in main text).

References

Althoff J, Grandjean C (1979). In vivo studies in Syrian golden hamsters: a transplacental bioassay of ten nitrosamines. *Natl Cancer Inst Monogr* (51):251-5.

Althoff J, Grandjean C, Gold B (1977). Diallylnitrosamine: a potent respiratory carcinogen in Syrian golden hamsters: brief communication. *J Natl Cancer Inst* **59**(5):1569-71.

Anderson EL and the U.S. Environmental Protection Agency Carcinogen Assessment Group (1983). Quantitative approaches in use to assess cancer risk. *Risk Analysis* **3**:277-295.

Anderson LM, Budinger JM, Maronpot RR, Good RA (1978). Transplacental lung tumorigenesis in the athymic mouse. *Cancer Res* **38** (1):137-41.

Anderson LM, Diwan BA, Fear NT, Roman E (2000). Critical windows of exposure for children's health: cancer in human epidemiological studies and neoplasms in experimental animal models. *Environ Health Perspect* **108 Suppl 3**:573-94.

Anderson LM, Hagiwara A, Kovatch RM, Rehm S, Rice JM (1989). Transplacental initiation of liver, lung, neurogenic, and connective tissue tumors by N-nitroso compounds in mice. *Fundam Appl Toxicol* **12**(3):604-20.

Armitage P, Doll R (1954). The age distribution of cancer and a multistage theory of carcinogenesis. *Br J Cancer* **8**:1-12.

Bailer AJ, Portier CJ (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* **44**: 417-431.

Barton HA, Cogliano VJ, Flowers L, Valcovic L, Setzer RW, Woodruff TJ (2005). Assessing susceptibility from early-life exposure to carcinogens. *Environ Health Perspect* **113**:1125-1133.

Baylin SB (2005). DNA methylation and gene silencing in cancer. *Nat Clin Pract Oncolo* **2 Suppl 1**:S4-11.

Bhatia S, Robison LL, Oberlin O, Greenberg M, Bunin G, Fossati-Bellani F *et al*. (1996). Breast cancer and other second neoplasm after childhood Hodgikin's disease. *N Eng J Med* **334**(12):745-51.

Birnbaum LS, Fenton SE (2003). Cancer and developmental exposure to endocrine disruptors. *Environ Health Perspect* **111**(4):389-94.

Boberg EW, Miller EC, Miller JA, Poland A, Liem A (1983). Strong evidence from studies with brachymorphic mice and pentachlorophenol that 1'-sulfooxysafrole is the major ultimate electrophilic and carcinogenic metabolite of 1'-hydroxysafrole in mouse liver. *Cancer Res* **43**(11):5163-73.

Bosch DA (1977). Short and long term effects of methyl- and ethylnitrosourea (MNU & ENU) on the developing nervous system of the rat. I. Long term effects: the induction of (multiple) gliomas. *Acta Neurol Scand* **55**(2):85-105.

Brittebo EB, Lindgren A, Tjalve H (1981). Fetal distribution and metabolism of N-nitrosodiethylamine in mice. *ACTA Pharmacol Toxicol;* **48 (4).** 355-363.

Calabrese EJ, Blain RB (1999). The single exposure carcinogen database: assessing the circumstances under which a single exposures carcinogen can cause cancer. *Tocicol Sci* **50**(2):169-85.

Cal/EPA, California Environmental Protection Agency (2004). *The California Environmental Protection Agency's Children's Environmental Health Program Biennial Report for 2002-2003. A report to the Governor and Legislature on Implementation of the Children's Environmental Health Initiative, including The Requirements of Chapter 731, Statutes of 1999, and The Requirements of Chapter 144, Statues of 2000.* Children's Environmental Health Center, Office of the Secretary, Cal/EPA, Sacramento California, January 1, 2004.

Cameron TP, Hickman RL, Kornreich MR, Tarone RE (1985). History, survival, and growth patterns of B6C3F1 mice and F344 rats in the National Cancer Institute Carcinogenesis Testing Program. *Fundam Appl Toxicol* **5**(3):526-38.

CancerChem (2000). Version 3.0. Electronic database of U.S. Public Health Service Publication No. 149, Survey of Compounds Which Have Been Tested for Carcinogenic Activity, covers literature from 1900 to 1998. GMA Industries, Inc.

Charles River Laboratories (1999). Technical Bulletin: Originally published as Technical Bulletion No. 1. **located at http://rats.info/flex_content_area/documents/rm_rm_n_techbul_spring_99.pdf**.

Chernozemski IN, Warnick GP (1970). Liver regeneration and induction of hepatomas in B6AF mice by urethane. *Cancer Res* **30**(11):2685-90.

Chhabra RS, Bucher JR, Haseman JK, Elwell MR, Kurtz PJ, Carlton BD (1993a). Comparative carcinogenicity of 5,5-diphenylhydantoin with or without perinatal exposure in rats and mice. *Fundam Appl Toxicol* **21**(2):174-86.

Chhabra RS, Bucher JR, Haseman JK, Elwell MR, Kurtz PJ, Carlton BD (1993b). Comparative carcinogenicity of polybrominated biphenyls with or without perinatal exposure in rats and mice. *Fundam Appl Toxicol* **21**(4):451-60.

Choudari Kommineni VR, Greenblatt M, Vesselinovitch SD, Mihailovich N (1970). Urethane carcinogenesis in rats: importance of age and dose. *J Natl Cancer Inst* **45**(4):687-96.

Crouch EA (1983). Uncertainties in interspecies extrapolations of carcinogenicity. *Environ Health Perspect* **50**: 321-327.

Crump KS, Hoel DG, Langley CH, Peto R (1976). Fundamental carcinogenic processes and their implications for low dose risk assessment. *Cancer Res* **36**(9 pt.1):2973-9.

Crump KS, Howe RB (1984). The mutistage model with a time-dependent dose pattern: Applications to carcinogenic risk assessment. *Risk Analysis* **4**:163-176.

Della Porta G, Dragani TA, Sozzi G (1987). Carcinogenic effects of infantile and long-term

2,3,7,8-tetrachlorodibenzo-p-dioxin treatment in the mouse. *Tumori* **73**(2): 99-107.

Diwan BA, Meier H, Huebner RJ (1974). Transplacental effects of 1-ethyl-1-nitrosourea in inbred strains of mice. IV. Rapid tumor induction in strain crosses. *J Natl Cancer Institute* **52**: 893-895.

Drinkwater NR, Ginsler JJ (1986). Genetic control of hepatocarcinogenesis in C57BL/6J and C3H/HeJ inbred mice. *Carcinogenesis* **7**(10):1701-7.

Druckrey H, Landschutz C (1971). Transplacental and neonatal carcinogenesis by ethylnitrosobiuret (ENBU) in BD IX-rats. *Z Krebsforsch Klin Onkol Cancer Res Clin Oncol* **76**(1):45-58.

Finkel AM (1995). A quantitative estimate of the variations in human susceptibility to cancer and its implications for risk management. *in: Olin S.S.; Farland W.; Park C.; Rhomberg L.; Scheuplein R.; Starr T. (Eds) Low-Dose Extrapolation of Cancer Risks: Issues and Perspectives,* pp. 297-328, ILSI Press, Washington D.C.

Finkel AM (2002). The joy before cooking: Preparing ourselves to write a risk research recipe. *Hum Ecol Risk Assess* **8**(6):1203-1221.

Fox JG, Cohen BJ, Loew R (1995). *Laboratory Animal Medicine*. Orlando, FL: Academic Press, Inc.

Ginsberg GL (2003). Assessing cancer risks from short-term exposures in children. *Risk Anal* **23**(1):19-34.

Grubbs CJ, Peckham JC, McDonough KD (1983). Effect of ovarian hormones on the induction of 1-methyl-1-nitrosourea- induced mammary cancer. *Carcinogenesis* **4**(4):495-7.

Harder JD, Stonebrook MJ, Pondy J (1993). Gestation and placentation in two New World Opossums: Didelphis virginiana and Mondodelphis domestica. *J Exp Zool* **266**(5):463-79.

Harkness JE, Wagner JE (1995). *The Biology and Medicine of Rabbits and Rodents*. Philadelphia, PA: Lea and Febiger.

Hattis D, Goble R, Chu M (2005). Age-related differences in susceptibility to carcinogenesis. II. Approaches for application and uncertainty analyses for individual genetically acting carcinogens. *Environ Health Perspect* **113**(4): 509-16.

Hattis D, Goble R, Russ A, Chu M, Ericson J (2004). Age-related differences in susceptibility to carcinogenesis: a quantitative analysis of empirical animal bioassay data. *Environ Health Perspect* **112**(11):1152-8.

Herbst AL, Ulfelder H, Poskanzer DC (1971). Adenocarcinoma of the vagina. Association of maternal stilbestrol therapy with tumor appearance in young women. *N Engl J Med* **284**(15):878-81.

Kauffman SL (1976). Susceptibility of fetal lung to transplacental 1-ethyl-1-nitrosourea: its relation to epithelial proliferation. *J Natl Cancer Inst* **57**(4):821-5.

Klein M (1959). Influence of low dose of 2-acetylaminofluorene on liver tumorigenesis in mice. *Proc Soc Exp Biol Med* **101**:637-8.

Lai CC, Miller JA, Miller EC, Liem A (1985). N-sulfooxy-2-aminofluorene is the major ultimate electrophilic and carcinogenic metabolite of N-hydroxy-2-acetylaminofluorene in the livers of infant male C57BL/6J x C3H/HeJ F1 (B6C3F1) mice. *Carcinogenesis* **6**(7):1037-45.

Maltoni C, Lefemine G, Ciliberti A, Cotti G, Carretti D (1981). Carcinogenicity bioassays of vinyl chloride monomer: a model of risk assessment on an experimental basis. *Environ Health Perspect* **41**:3-29.

Martin MS, Martin F, Justrabo E, Knopf JF, Bastien H, Knobel S (1974). [Induction of colonic cancers in rats through a single injection of 1,2 dimethylhydrazine]. *Biol Gastroenterol (Paris)* **7**(1):37-42.

McConnell EE (1992). Comparative responses in carcinogenesis bioassays as a function of age at first exposure. *in: Guzelian, P.S.; Henry, C.J.; Olin, S.S. (Eds) Similarities and Differences Between Children and Adults: Implications for Risk Assessment*. ILSI Press, Washington, D.C.

Meranze DR, Gruenstein M, Shimkin MB (1969). Effect of age and sex on the development of neoplasms in Wistar rats receiving a single intragastric instillation of 7,13-Dimethylbenz(a)anthracene. *Int J Cancer* **4**(4):480-6.

Merck (1998). *The Merck Veterinary Manual 8th Edition*. Merck & Co., Inc., Whitehouse Station.

Miller MD, Marty MA, Arcus A, Brown J, Morry D, Sandy M (2002). Differences between children and adults: implications for risk assessment at California EPA. *Int J Toxicol* **21**:403-418.

Mohr U, Althoff J (1965). [The transplacental action of the carcinogen diethylnitrosamine in the mouse]. *Z Krebsforsch* **67**(2):152-5.

Mohr U, Emura M, Kamino K, Steinmann J, Kohler M, Morawietz G *et al*. (1995). Increased risk of cancer in the descendants of Syrian hamsters exposed prenatally to diethylnitrosamine (DEN). *Int J Cancer* **63**(1):86-91.

Mohr U, Reznik-Schuller H, Reznik G, Hilfrich J (1975). Transplacental effects of diethylnitrosamine in Syrian hamsters as related to different days of administration during pregnancy. *J Natl Cancer Inst* **55** (3):681-3.

Moysich KB, Menezes RJ, Michalek AM (2002). Chernobyl-related ionising radiation exposure and cancer risk: an epidemiological review. *Lancet Oncol* **3**(5):269-79.

Murdoch DJ, Krewski D, Wargo J. (1992). Cancer risk assessment with intermittent exposure. *Risk Anal* 12(4*):* 569-577.

Naito M, Aoyama H, Fujioka Y, Ito A (1985). Induction of gliomas in Mongolian gerbils (Meriones unguiculatus) following neonatal administration of N-ethyl-N-nitrosourea. *J Natl Cancer Inst* **75**(3):581-7.

Naito M, Naito Y, Ito A (1981). Effect of age at treatment on the incidence and location of neurogenic tumors induced in Wistar rats by a single dose of N-ethyl-N-nitrosourea. *Gann* **72**(4):569-77.

Naito M, Naito Y, Ito A (1982). Effect of phenobarbital on the development of tumors in mice treated neonatally with N-ethyl-N-nitrosourea. *Gann* **73**(1):111-4.

Napalkov NP, Rice JM, Tomatis L, Yamasaki H (1989). Perinatal and multigenerational carcinogenesis. *IARC Sci Publ*. **vol 96**, International Agency for Research on Cancer, Lyon France.

Noronha RF, Goodall CM (1984). The effects of estrogen on single dose dimethylnitrosamine carcinogenesis in male inbred Crl/CDF rats. *Carcinogenesis* **5**(8):1003-7.

NRC, National Research Council (1990). Health effects of exposure to low levels of ionizing radiation. BEIR V, Committee on the Biological Effects of Ionizing Radiation. Washington, D.C.: National Academy Press.

NRC, National Research Council (1993). *Pesticides in the diets of infants and children*. Washington, D.C.: National Academy Press.

NRC, National Research Council (1994). Science and Judgment in Risk Assessment. Committee on Risk Assessment of Hazardous Air Pollutants, Commission on Life Sciences. Washington, D.C.: National Academy Press.

Penn I (2000). Post-transplant malignancy: the role of immunosuppression. *Drug* **23. 23**(2. 2):101-13, 101-13.

Pereira MA, Knutsen GL, Herren-Freund SL (1985). Effect of subsequent treatment of chloroform or phenobarbital on the incidence of liver and lung tumors initiated by ethylnitrosourea in 15 day old mice. *Carcinogenesis* **6**(2):203-7.

Peto R (1978). Carcinogenic effects of chronic exposure to very low levels of toxic substances. *Environ Health Perspect* **22**:155-9.

Poiley SM (1972). Growth tables for 66 strains and stocks of laboratory animals. *Lab Anim Sci* **22**(5):758-79.

Preston-Martin S (1989). Epidemiological studies of perinatal carcinogenesis. *IARC Sci Publ*. **96**:289-314, International Agency for Research on Cancer, Lyon France.

Rao KV, Vesselinovitch SD (1973). Age- and sex-associated diethylnitrosamine dealkylation activity of the mouse liver and hepatocarcinogenesis. *Cancer Res* **33**(7):1625-7.

Rice, JM (1979). Problems and perspectives in perinatal carcinogenesis: a summary of the conference. Natl Cancer Inst Monogr 51:271-278.

Rice JM, Rehm S, Donovan PJ, Perantoni AO (1989). Comparative transplacental carcinogenesis by directly acting and metabolism-dependent alkylating agents in rodents and nonhuman primates. *IARC Sci Publ* (96):17-34.

Schmahl W (1988). Synergistic induction of tumors in NMRI mice by combined fetal X-irradiation with low doses and ethylnitrosourea administered to juvenile offspring. *Carcinogenesis* **9**(8):1493-8.

Searle CE, Jones EL (1976). The multipotential carcinogenic action of N-ethyl-N-nitrosourea administered neonatally to mice. *Br J Cancer* **33**(6):612-25.

Slikker W, III, Mei N, Chen T. (2004). N-ethyl-N-nitrosourea (ENU) increased brain mutation in prenatal and neonatal mice but not in adults. Tox. Sci **81**., 112-120

Terracini B, Testa MC (1970). Carcinogenicity of a single administration of N-nitrosomethylurea: a comparison between newborn and 5-week-old mice and rats. *Br J Cancer* **24**(3):588-98.

Terracini B, Testa MC, Cabral JR, Rossi L (1976). The roles of age at treatment and dose in carcinogenesis in C3Hf/Dp mice with a single administration of N-nitroso-N-methylurea. *Br J Cancer* **33**(4):427-39.

Tomatis L, Turusov V, Guibbert D, Duperray B, Malaveille C, Pacheco H (1971). Transplacental carcinogenic effect of 3-methylcholanthrene in mice and its quantitation in fetal tissues. *J Natl Cancer Inst* **47**(3):645-51.

Tomatis L, Ponomarkov V, Turusov V (1977). Effects of ethylnitrosoura administration during pregnancy on three subsequent generation of BDVI rats. *Int J Cancer* **19**:240-8.

Toth B (1968). A critical review of experiments in chemical carcinogenesis using newborn animals. *Cancer Res* **28**:727-738.

Truhaut R, Lesca P, Dechambre RP, Gerard-Marchant R (1966). [On the means of manifestation of the cancerogenic power of 3,4-benzopyrene in newborn or treated immediately after weaning mice]. *Pathol Biol* **14**(19):955-9.

Turusov V, Tomatis L, Guibberi D, Duperray B, Pacheco H. (1973). The effect of prenatal exposure of mice to methyl cholanthrene combined with the neonatal administration of diethylnitrosamine. *In: Tomatis, L. And U. Mohr (ED.). International Agency for Research on Cancer Scientific Publication, No 4. Transplacental Carcinogenesis. Proceeding of a Meeting, Hannover, West Germany, Oct 6-7, 1971. XII pp. 84-91. International Agency for Research on Cancer: Lyon, France .*

Turusov VS, Trukhanova LS, Parfenov YuD, Tomatis L (1992). Occurrence of tumours in the descendants of CBA male mice prenatally treated with diethylstilbestrol. *Int J Cancer* **50**(1):131-5.

U.S. Environmental Protection Agency (U.S. EPA) (2005). *Supplemental Guidance for Assessing Cancer Susceptibility from Early-Life Exposure to Carcinogens*. EPA/630/R-03/003F, March 2005.

Vesselinovitch SD (1980). Infant mouse as a sensitive bioassay system for carcinogenicity of N-nitroso compounds. *IARC Sci Publ* (31):645-55.

Vesselinovitch SD (1983). Perinatal hepatocarcinogenesis. *Biol Res Pregnancy Perinatol* **4**(1):22-5.

Vesselinovitch SD, Itze L, Mihailovich N, Rao KV, Manojlovski B (1973). Role of hormonal environment, partial hepatectomy, and dose of ethylnitrosourea in renal carcinogenesis. *Cancer Res* **33**(2):339-41.

Vesselinovitch SD, Koka M, Mihailovich N, Rao KV (1984). Carcinogenicity of diethylnitrosamine in newborn, infant, and adult mice. *J Cancer Res Clin Oncol* **108**(1):60-5.

Vesselinovitch SD, Koka M, Rao KV, Mihailovich N, Rice JM (1977). Prenatal multicarcinogenesis by ethylnitrosourea in mice. *Cancer Res* **37**(6):1822-8.

Vesselinovitch SD, Kyriazis AP, Mihailovich N, Rao KV (1975a). Conditions modifying development of tumors in mice at various sites by benzo(a)pyrene. *Cancer Res* **35**(11 Pt 1):2948-53.

Vesselinovitch SD, Rao KV, Mihailovich N (1975b). Factors modulating benzidine carcinogenicity bioassay. *Cancer Res* **35**(10):2814-9.

Vesselinovitch SD, Rao KV, Mihailovich N (1979a). Neoplastic response of mouse tissues during perinatal age periods and its significance in chemical carcinogenesis. *Natl Cancer Inst Monogr* (51):239-50.

Vesselinovitch SD, Rao KV, Mihailovich N (1979b). Transplacental and lactational carcinogenesis by safrole. *Cancer Res* **39**(11):4378-80

Vesselinovitch SD, Rao KV, Mihailovich N, Rice JM, Lombard LS (1974). Development of broad spectrum of tumors by ethylnitrosourea in mice and the modifying role of age, sex, and strain. *Cancer Res* **34**(10):2530-8.

Walters MA (1966). The induction of lung tumours by the injection of 9,10-dimethyl-1,2-benzanthracene (DMBA) into newborn suckling and young adult mice. A dose response study. *Br J Cancer* **20**(1):148-60.

Wiggenhauser A, Schmahl W (1987). Postnatal development and neoplastic disease pattern in NMRI mice after combined treatment with ethylnitrosourea and X-irradiation on different days of the fetal period. *Int J Radiat Biol Relat Stud Phys Chem Med* **51**(6):1021-9.

Wood M, Flaks A, Clayson DB (1970). The carcinogenic activity of dibutylnitrosamine in IF x C57 mice. *Eur J Cancer* **6**(5):433-40.

Zeller WJ, Ivankovic S, Zeller J (1978). Induction of malignant tumors in Wistar and Sprague-Dawley rats by single doses of n-butyl-nitrosourea in perinatal and juvenile phases of development. *Arch Geschwulstforsch* **48**(1):9-16.

Appendices

A. Default Body Weights for Rats and Mice During the First Six Months of Life

B. Unadjusted ASFs for Multi-Window Studies

Prenatal Exposure Window

Postnatal Exposure Window

Juvenile Exposure Window

C. ASF Mixture Frequency Distributions from Methods 1-3 for Multi-Window Studies

Prenatal Exposure Window

Postnatal Exposure Window

Juvenile Exposure Window

D. DEN Case Study: Cancer Potency Distributions for DEN Experiments

E. ENU Case Study: Cancer Potency Distributions for ENU Experiments

F. Early Life Across-Window Studies of Two Non-Genotoxic Carcinogens

Appendix A

Default Body Weights for Rats and Mice During the First Six Months of Life

This appendix describes the approach taken to calculate body weights when needed for dose calculations. For example, doses in the postnatal and juvenile windows may have been reported as bolus amounts administered (e.g., milligrams) and the publication may not have reported the weight of the animals on the day of compound administration. Because in neonatal and juvenile rodents, body weight changes rapidly through development, default body weights for the first six months of life (i.e., day 1-168) were estimated for each postnatal day for mice and rats, for use in calculating dose in mg/kg-bd wt when body weight on the day of dosing was not reported.

Growth Model Applied

When standard growth models were applied to the data (e.g., models of Richards, Gompertz, and Janoschek), most seemed to overpredict body weight at very young ages. Thus, OEHHA applied a more flexible model, which was constrained to pass through the actual data point for the day 1 body weight. The modeling was performed using constrained linear regression using the statistical package, STATA (Stata Corp, College Station, Texas). The model takes the form:

$$\text{BodyWeight}_{age} = \beta_0 + \beta_1 (\text{day-1}) + \beta_2 (\text{day-1})^2 + \beta_3 (\text{day-1})^3 + \beta_4 (\text{day-1})^4 \qquad \text{(Eqn. 1)}$$

where β_0 is defined as the measured average body weight on day 1 of life (i.e., redefining day 1 as 'day 0' or the origin). The variable day is the day of life, and parameters, β_1, β_2, β_3, β_4 are estimated. Fitted values for each day of life through six months of age (i.e., day 168) are provided in look up tables, which are appended.

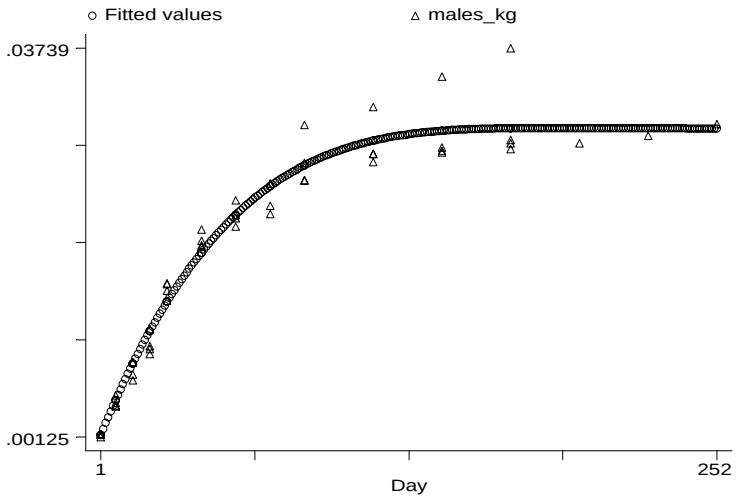
Mice

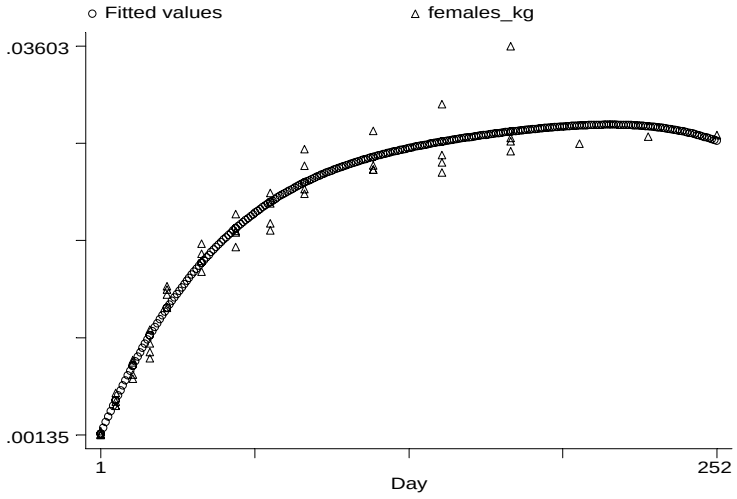
Default body weights were estimated using data from a survey of Poiley (1972) for several strains of mice. Data from BALB/cANCr, AKR/LwCr and C57Bl/6Cr mice were selected for use in deriving the default value, as these datasets comprised the largest numbers of animals surveyed (i.e., early life groups represented averages of 256 to 547 mice for each species). Table

A7 gives the data used in the model fitting. Body weights for all three species were quite similar during the first 70 days of life. The AKR/LwCr mice became heavier than the other two species later in life, thus taken together data from these three strains likely provide a reasonable average.

Figure A1 displays the model fit for data from BALB/c, C57Bl/6Cr, AKR/LwCr, and DBA/2Cr mouse strains. Two plots are shown. The first plot shows the data and model fit for male mice, and the second plot does the same for female mice.

Figure A1. Model Fitted Data for Male and Female Mice





Tables A1 and A2 give the default day-specific body weight values for male and female mice based on these model fits.

Table A1. Male Mice: Default Body Weight for the First 168 Days of Life

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00144	44	0.01894	87	0.02684	130	0.02950
2	0.00199	45	0.01921	88	0.02695	131	0.02953
3	0.00254	46	0.01948	89	0.02705	132	0.02955
4	0.00308	47	0.01974	90	0.02715	133	0.02958
5	0.00361	48	0.02000	91	0.02725	134	0.02960
6	0.00414	49	0.02026	92	0.02735	135	0.02962
7	0.00465	50	0.02050	93	0.02744	136	0.02964
8	0.00516	51	0.02075	94	0.02753	137	0.02966
9	0.00566	52	0.02099	95	0.02762	138	0.02968
10	0.00616	53	0.02122	96	0.02771	139	0.02970
11	0.00664	54	0.02145	97	0.02779	140	0.02972
12	0.00712	55	0.02168	98	0.02787	141	0.02974
13	0.00759	56	0.02190	99	0.02795	142	0.02975
14	0.00806	57	0.02212	100	0.02803	143	0.02977
15	0.00851	58	0.02233	101	0.02811	144	0.02978
16	0.00896	59	0.02254	102	0.02818	145	0.02980
17	0.00940	60	0.02274	103	0.02825	146	0.02981
18	0.00984	61	0.02294	104	0.02832	147	0.02982
19	0.01027	62	0.02313	105	0.02839	148	0.02983
20	0.01069	63	0.02333	106	0.02845	149	0.02984
21	0.01110	64	0.02351	107	0.02851	150	0.02985
22	0.01151	65	0.02370	108	0.02857	151	0.02986
23	0.01191	66	0.02388	109	0.02863	152	0.02987
24	0.01231	67	0.02405	110	0.02869	153	0.02988
25	0.01270	68	0.02422	111	0.02874	154	0.02989
26	0.01308	69	0.02439	112	0.02880	155	0.02990
27	0.01345	70	0.02456	113	0.02885	156	0.02990
28	0.01382	71	0.02472	114	0.02890	157	0.02991
29	0.01419	72	0.02487	115	0.02895	158	0.02992
30	0.01454	73	0.02503	116	0.02900	159	0.02992
31	0.01490	74	0.02518	117	0.02904	160	0.02993
32	0.01524	75	0.02532	118	0.02908	161	0.02993
33	0.01558	76	0.02547	119	0.02913	162	0.02994
34	0.01591	77	0.02561	120	0.02917	163	0.02994
35	0.01624	78	0.02575	121	0.02921	164	0.02994
36	0.01656	79	0.02588	122	0.02924	165	0.02995
37	0.01688	80	0.02601	123	0.02928	166	0.02995
38	0.01719	81	0.02614	124	0.02932	167	0.02995
39	0.01749	82	0.02626	125	0.02935	168	0.02996
40	0.01779	83	0.02638	126	0.02938		
41	0.01809	84	0.02650	127	0.02941		
42	0.01838	85	0.02662	128	0.02944		
43	0.01866	86	0.02673	129	0.02947		

Table A2. Female Mice: Default Body Weight for the First 168 Days of Life

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00147	44	0.01719	87	0.02418	130	0.02710
2	0.00198	45	0.01742	88	0.02428	131	0.02715
3	0.00248	46	0.01766	89	0.02438	132	0.02719
4	0.00298	47	0.01789	90	0.02447	133	0.02723
5	0.00346	48	0.01812	91	0.02457	134	0.02727
6	0.00394	49	0.01834	92	0.02466	135	0.02732
7	0.00441	50	0.01856	93	0.02475	136	0.02736
8	0.00488	51	0.01877	94	0.02484	137	0.02740
9	0.00533	52	0.01898	95	0.02493	138	0.02744
10	0.00578	53	0.01918	96	0.02501	139	0.02748
11	0.00622	54	0.01939	97	0.02509	140	0.02752
12	0.00665	55	0.01958	98	0.02518	141	0.02755
13	0.00708	56	0.01978	99	0.02526	142	0.02759
14	0.00750	57	0.01997	100	0.02533	143	0.02763
15	0.00791	58	0.02015	101	0.02541	144	0.02766
16	0.00832	59	0.02033	102	0.02549	145	0.02770
17	0.00872	60	0.02051	103	0.02556	146	0.02774
18	0.00911	61	0.02069	104	0.02563	147	0.02777
19	0.00949	62	0.02086	105	0.02570	148	0.02781
20	0.00987	63	0.02103	106	0.02577	149	0.02784
21	0.01024	64	0.02119	107	0.02584	150	0.02787
22	0.01061	65	0.02135	108	0.02591	151	0.02791
23	0.01097	66	0.02151	109	0.02597	152	0.02794
24	0.01132	67	0.02167	110	0.02604	153	0.02797
25	0.01167	68	0.02182	111	0.02610	154	0.02800
26	0.01201	69	0.02197	112	0.02616	155	0.02804
27	0.01234	70	0.02211	113	0.02622	156	0.02807
28	0.01267	71	0.02226	114	0.02628	157	0.02810
29	0.01299	72	0.02240	115	0.02634	158	0.02813
30	0.01331	73	0.02253	116	0.02640	159	0.02816
31	0.01362	74	0.02267	117	0.02645	160	0.02819
32	0.01393	75	0.02280	118	0.02651	161	0.02822
33	0.01423	76	0.02293	119	0.02656	162	0.02825
34	0.01452	77	0.02305	120	0.02662	163	0.02827
35	0.01481	78	0.02318	121	0.02667	164	0.02830
36	0.01509	79	0.02330	122	0.02672	165	0.02833
37	0.01537	80	0.02342	123	0.02677	166	0.02836
38	0.01565	81	0.02353	124	0.02682	167	0.02838
39	0.01592	82	0.02365	125	0.02687	168	0.02841
40	0.01618	83	0.02376	126	0.02692		
41	0.01644	84	0.02387	127	0.02696		
42	0.01669	85	0.02397	128	0.02701		
43	0.01694	86	0.02408	129	0.02706		

Rats

Default body weights applicable to all rat strains except Sprague-Dawley rats were estimated using data from surveys by Poiley (1972) and Cameron et al. (1985) for Fischer 344 (F344) rats (See Table A8). The body weights of F344 rats are reasonably representative of most other rat strains (U.S. EPA, 1988). Data from Sprague-Dawley rats, which become much heavier than most other rat strains, were used to estimate default body weights for this strain using normative data surveyed by Poiley (1972) (See Table A9). Figure A2 displays the model fit for data from the F344 rat strain. The first plot shows the fit for males, the second for females. Figure A3 displays the model fit for data from the Sprague-Dawley rat strain. The first plot shows the fit for males, the second for females.

Figure A2. Model Fitted Data for Male and Female F344 Rats

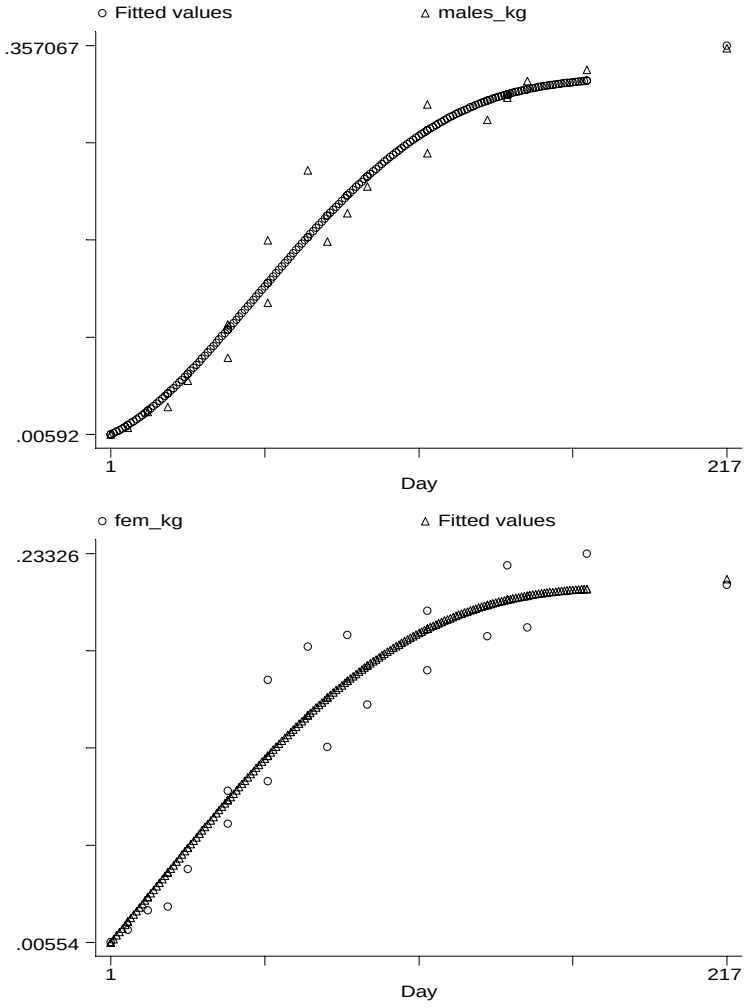
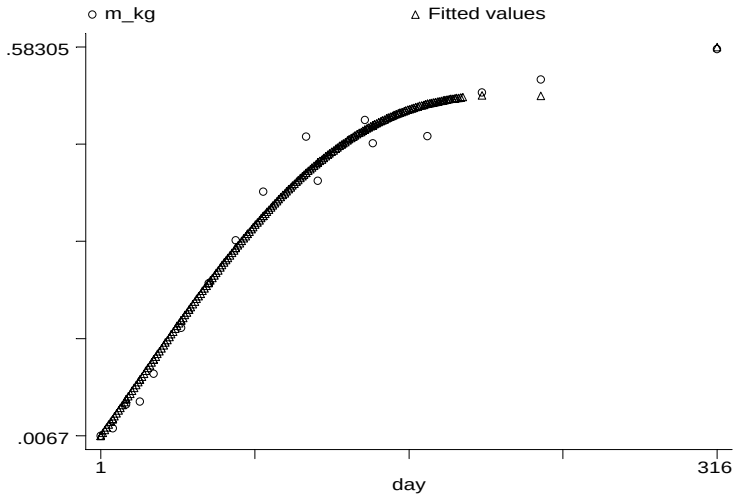
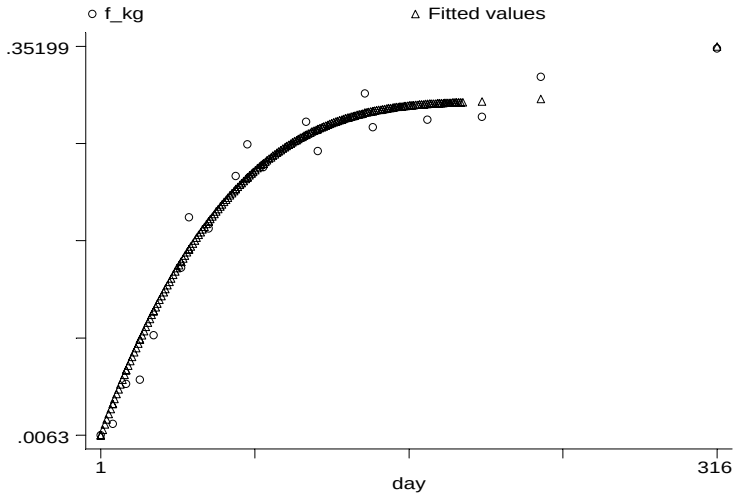


Figure A3. Model Fitted Data for Male and Female Sprague-Dawley Rats





Tables A3 and A4 give the default day-specific body weight values for male and female rats (with the exception of Sprague-Dawley rats) based on these model fits. The default day-specific body weight values for male and female Sprague-Dawley rats were based on model fits derived from data specific to Sprague-Dawley rats. These values are shown in Tables A5 and A6.

Table A3. Male Rats: Default Body Weight for the First 168 Days of Life (based on F344 Rats; default does not apply to Sprague-Dawley rats)

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00592	44	0.10643	87	0.22932	130	0.30443
2	0.00712	45	0.10943	88	0.23176	131	0.30544
3	0.00839	46	0.11244	89	0.23416	132	0.30641
4	0.00975	47	0.11545	90	0.23654	133	0.30736
5	0.01118	48	0.11847	91	0.23888	134	0.30828
6	0.01268	49	0.12150	92	0.24120	135	0.30916
7	0.01426	50	0.12452	93	0.24348	136	0.31002
8	0.01591	51	0.12755	94	0.24573	137	0.31085
9	0.01762	52	0.13058	95	0.24795	138	0.31165
10	0.01940	53	0.13361	96	0.25014	139	0.31242
11	0.02125	54	0.13664	97	0.25230	140	0.31316
12	0.02315	55	0.13966	98	0.25442	141	0.31387
13	0.02512	56	0.14268	99	0.25651	142	0.31456
14	0.02714	57	0.14570	100	0.25857	143	0.31523
15	0.02923	58	0.14871	101	0.26059	144	0.31586
16	0.03136	59	0.15171	102	0.26258	145	0.31648
17	0.03355	60	0.15471	103	0.26454	146	0.31706
18	0.03579	61	0.15769	104	0.26646	147	0.31763
19	0.03808	62	0.16067	105	0.26835	148	0.31817
20	0.04042	63	0.16363	106	0.27021	149	0.31869
21	0.04280	64	0.16658	107	0.27203	150	0.31919
22	0.04523	65	0.16952	108	0.27382	151	0.31966
23	0.04769	66	0.17245	109	0.27557	152	0.32012
24	0.05020	67	0.17536	110	0.27728	153	0.32056
25	0.05275	68	0.17826	111	0.27897	154	0.32098
26	0.05533	69	0.18114	112	0.28061	155	0.32138
27	0.05796	70	0.18400	113	0.28223	156	0.32176
28	0.06061	71	0.18684	114	0.28381	157	0.32213
29	0.06330	72	0.18967	115	0.28535	158	0.32248
30	0.06601	73	0.19247	116	0.28686	159	0.32282
31	0.06876	74	0.19526	117	0.28833	160	0.32314
32	0.07153	75	0.19802	118	0.28977	161	0.32345
33	0.07433	76	0.20077	119	0.29118	162	0.32375
34	0.07716	77	0.20349	120	0.29255	163	0.32404
35	0.08000	78	0.20619	121	0.29389	164	0.32432
36	0.08287	79	0.20886	122	0.29519	165	0.32458
37	0.08576	80	0.21151	123	0.29646	166	0.32485
38	0.08867	81	0.21413	124	0.29770	167	0.32510
39	0.09159	82	0.21673	125	0.29890	168	0.32535
40	0.09454	83	0.21930	126	0.30007		
41	0.09749	84	0.22185	127	0.30121		
42	0.10046	85	0.22437	128	0.30231		
43	0.10344	86	0.22686	129	0.30339		

Table A4. Female Rats: Default Body Weight for the First 168 Days of Life (based on F344 Rats; default does not apply to Sprague-Dawley rats)

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00554	44	0.09273	87	0.16280	130	0.20171
2	0.00756	45	0.09465	88	0.16408	131	0.20224
3	0.00959	46	0.09655	89	0.16534	132	0.20276
4	0.01162	47	0.09844	90	0.16658	133	0.20325
5	0.01365	48	0.10032	91	0.16780	134	0.20374
6	0.01570	49	0.10219	92	0.16901	135	0.20420
7	0.01774	50	0.10405	93	0.17020	136	0.20466
8	0.01980	51	0.10589	94	0.17137	137	0.20509
9	0.02185	52	0.10772	95	0.17253	138	0.20552
10	0.02391	53	0.10955	96	0.17366	139	0.20593
11	0.02597	54	0.11136	97	0.17478	140	0.20632
12	0.02803	55	0.11315	98	0.17588	141	0.20670
13	0.03010	56	0.11494	99	0.17696	142	0.20707
14	0.03216	57	0.11671	100	0.17802	143	0.20743
15	0.03423	58	0.11846	101	0.17907	144	0.20777
16	0.03630	59	0.12021	102	0.18010	145	0.20810
17	0.03836	60	0.12194	103	0.18111	146	0.20841
18	0.04043	61	0.12365	104	0.18210	147	0.20871
19	0.04250	62	0.12535	105	0.18307	148	0.20900
20	0.04456	63	0.12704	106	0.18403	149	0.20928
21	0.04662	64	0.12871	107	0.18496	150	0.20955
22	0.04869	65	0.13037	108	0.18588	151	0.20980
23	0.05074	66	0.13202	109	0.18679	152	0.21005
24	0.05280	67	0.13364	110	0.18767	153	0.21028
25	0.05485	68	0.13526	111	0.18853	154	0.21050
26	0.05690	69	0.13685	112	0.18938	155	0.21071
27	0.05894	70	0.13843	113	0.19021	156	0.21091
28	0.06098	71	0.14000	114	0.19103	157	0.21111
29	0.06302	72	0.14155	115	0.19182	158	0.21129
30	0.06505	73	0.14308	116	0.19260	159	0.21146
31	0.06707	74	0.14460	117	0.19336	160	0.21162
32	0.06909	75	0.14610	118	0.19410	161	0.21178
33	0.07111	76	0.14759	119	0.19483	162	0.21192
34	0.07311	77	0.14905	120	0.19554	163	0.21206
35	0.07511	78	0.15051	121	0.19623	164	0.21219
36	0.07710	79	0.15194	122	0.19691	165	0.21232
37	0.07909	80	0.15336	123	0.19756	166	0.21243
38	0.08106	81	0.15476	124	0.19821	167	0.21254
39	0.08303	82	0.15614	125	0.19883	168	0.21264
40	0.08499	83	0.15751	126	0.19944		
41	0.08694	84	0.15886	127	0.20003		
42	0.08888	85	0.16019	128	0.20061		
43	0.09081	86	0.16150	129	0.20117		

Table A5. Male Sprague-Dawley Rats: Default Body Weight for the First 168 Days of Life

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00670	44	0.17648	87	0.35543	130	0.42990
2	0.00877	45	0.18129	88	0.35848	131	0.43110
3	0.01099	46	0.18611	89	0.36149	132	0.42985
4	0.01334	47	0.19091	90	0.36425	133	0.43218
5	0.01583	48	0.19570	91	0.36715	134	0.43204
6	0.01845	49	0.20048	92	0.37000	135	0.43144
7	0.02120	50	0.20525	93	0.37257	136	0.43240
8	0.02406	51	0.21000	94	0.37529	137	0.43290
9	0.02705	52	0.21473	95	0.37792	138	0.43292
10	0.03015	53	0.21944	96	0.38048	139	0.43448
11	0.03336	54	0.22413	97	0.38276	140	0.43355
12	0.03668	55	0.22880	98	0.38515	141	0.43415
13	0.04009	56	0.23343	99	0.38745	142	0.43426
14	0.04361	57	0.23804	100	0.38986	143	0.43590
15	0.04721	58	0.24271	101	0.39196	144	0.43502
16	0.05091	59	0.24713	102	0.39396	145	0.43565
17	0.05469	60	0.25164	103	0.39565	146	0.43578
18	0.05856	61	0.25623	104	0.39903	147	0.43540
19	0.06250	62	0.26049	105	0.40008	148	0.43651
20	0.06652	63	0.26503	106	0.40283	149	0.43711
21	0.07061	64	0.26944	107	0.40322	150	0.43718
22	0.07476	65	0.27370	108	0.40530	151	0.43672
23	0.07898	66	0.27803	109	0.40704	152	0.43774
24	0.08326	67	0.28221	110	0.40844	153	0.43823
25	0.08760	68	0.28644	111	0.40949	154	0.43817
26	0.09198	69	0.29051	112	0.41220	155	0.43756
27	0.09642	70	0.29462	113	0.41254	156	0.43842
28	0.10091	71	0.29856	114	0.41454	157	0.43872
29	0.10544	72	0.30254	115	0.41616	158	0.44047
30	0.11001	73	0.30655	116	0.41742	159	0.43964
31	0.11461	74	0.31037	117	0.41831	160	0.44026
32	0.11925	75	0.31422	118	0.41881	161	0.44030
33	0.12392	76	0.31787	119	0.42095	162	0.44178
34	0.12862	77	0.32173	120	0.42270	163	0.44268
35	0.13334	78	0.32540	121	0.42203	164	0.44298
36	0.13808	79	0.32887	122	0.42299	165	0.44270
37	0.14285	80	0.33253	123	0.42556	166	0.44383
38	0.14762	81	0.33598	124	0.42571	167	0.44435
39	0.15242	82	0.33922	125	0.42545	168	0.44629
40	0.15722	83	0.34263	126	0.42678		
41	0.16203	84	0.34603	127	0.42770		
42	0.16684	85	0.34920	128	0.42819		
43	0.17166	86	0.35233	129	0.42825		

Table A6. Female Sprague-Dawley Rats: Default Body Weight for the First 168 Days of Life

Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)	Day of life	Body weight (kg)
1	0.00630	44	0.15144	87	0.25154	130	0.28142
2	0.00966	45	0.15449	88	0.25302	131	0.28167
3	0.01304	46	0.15752	89	0.25446	132	0.28101
4	0.01643	47	0.16051	90	0.25580	133	0.28167
5	0.01984	48	0.16348	91	0.25717	134	0.28140
6	0.02326	49	0.16642	92	0.25850	135	0.28097
7	0.02670	50	0.16933	93	0.25972	136	0.28109
8	0.03015	51	0.17221	94	0.26098	137	0.28104
9	0.03360	52	0.17505	95	0.26219	138	0.28081
10	0.03706	53	0.17787	96	0.26336	139	0.28114
11	0.04054	54	0.18065	97	0.26440	140	0.28054
12	0.04401	55	0.18339	98	0.26548	141	0.28050
13	0.04749	56	0.18611	99	0.26650	142	0.28028
14	0.05098	57	0.18879	100	0.26755	143	0.28061
15	0.05446	58	0.19146	101	0.26848	144	0.28001
16	0.05795	59	0.19403	102	0.26935	145	0.27996
17	0.06144	60	0.19660	103	0.27009	146	0.27972
18	0.06492	61	0.19918	104	0.27144	147	0.27928
19	0.06840	62	0.20162	105	0.27192	148	0.27940
20	0.07188	63	0.20414	106	0.27301	149	0.27931
21	0.07535	64	0.20658	107	0.27323	150	0.27903
22	0.07882	65	0.20896	108	0.27405	151	0.27855
23	0.08228	66	0.21133	109	0.27473	152	0.27861
24	0.08573	67	0.21362	110	0.27527	153	0.27847
25	0.08917	68	0.21592	111	0.27567	154	0.27813
26	0.09260	69	0.21813	112	0.27668	155	0.27758
27	0.09602	70	0.22034	113	0.27680	156	0.27757
28	0.09942	71	0.22247	114	0.27751	157	0.27735
29	0.10281	72	0.22458	115	0.27808	158	0.27766
30	0.10619	73	0.22669	116	0.27850	159	0.27702
31	0.10955	74	0.22871	117	0.27877	160	0.27690
32	0.11290	75	0.23072	118	0.27889	161	0.27658
33	0.11623	76	0.23264	119	0.27960	162	0.27678
34	0.11954	77	0.23462	120	0.28016	163	0.27676
35	0.12283	78	0.23650	121	0.27982	164	0.27653
36	0.12610	79	0.23829	122	0.28007	165	0.27608
37	0.12935	80	0.24014	123	0.28090	166	0.27614
38	0.13257	81	0.24189	124	0.28082	167	0.27599
39	0.13578	82	0.24354	125	0.28059	168	0.27635
40	0.13896	83	0.24524	126	0.28094		
41	0.14212	84	0.24691	127	0.28112		
42	0.14525	85	0.24849	128	0.28114		
43	0.14836	86	0.25003	129	0.28099		

Table A7. Mouse Data Used in Fitting Eqn. 1 (Source: Poiley, 1972)

Strain	Day	Bodyweight (kg)		Strain	Day	Bodyweight (kg)	
		Males	Females			Males	Females
Balb/C	1	0.00125	0.00169	DBA/2Cr	1	0.00148	0.00135
	7	0.00511	0.00509		7	0.00406	0.00396
	14	0.00817	0.00803		14	0.00823	0.00781
	21	0.01127	0.01076		21	0.00969	0.00952
	28	0.01545	0.01432		28	0.01554	0.01462
	42	0.01948	0.0169		42	0.01908	0.01752
	56	0.02082	0.01941		56	0.02188	0.01958
	70	0.02197	0.02022		70	0.02481	0.02202
	84	0.02516	0.02287		84	0.02672	0.02535
	112	0.0276	0.02504		112	0.02682	0.02541
	140	0.02816	0.02476		140	0.02788	0.02565
	168	0.02857	0.02667		168	0.02886	0.02754
	196	0.02857	0.02735				
	224	0.02925	0.02798				
	252	0.03033	0.0281				
AKR/LwCr	1	0.00153	0.00143				
	7	0.00444	0.0043				
	14	0.00704	0.00674				
	21	0.00896	0.00874				
	28	0.01391	0.0127				
	42	0.02053	0.01841				
	56	0.02327	0.02105				
	70	0.02481	0.02296				
	84	0.03028	0.02686				
	112	0.03193	0.02848				
	140	0.03477	0.03087				
	168	0.03739	0.03603				
C57Bl/6Cr	1	0.00149	0.0014				
	7	0.00419	0.00394				
	14	0.00653	0.00637				
	21	0.00941	0.00818				
	28	0.01486	0.01389				
	42	0.01893	0.01592				
	56	0.02159	0.01812				
	70	0.02276	0.0196				
	84	0.02509	0.02328				
	112	0.02756	0.02503				
	140	0.02771	0.02632				
	168	0.02803	0.02787				

Table A8. F344 Rat Data Used in Fitting Eqn. 1

Age (in days)	Bodyweight (kg)		Reference
	Males	Females	
1	0.00592	0.00574	Poiley, 1972
7	0.01201	0.01285	
14	0.02634	0.02436	
21	0.0307	0.02658	
28	0.05423	0.04849	
42	0.10506	0.09446	
56	0.18112	0.15936	
70	0.24446	0.17893	
84	0.20588	0.18567	
112	0.3042	0.19976	
140	0.31301	0.22657	
168	0.33542	0.23326	
42	0.075	0.075	Cameron et al., 1985
56	0.125	0.1	
77	0.18	0.12	
91	0.23	0.145	
112	0.26	0.165	
133	0.29	0.185	
140	0.31	--	
147	0.325	0.19	
217	0.355	0.215	

Table A9. Sprague-Dawley Rat Data Used in Fitting Eqn. 1 (Source: Poiley, 1972)

Age (in days)	Bodyweight (kg)	
	Males	Females
1	0.0067	0.0063
7	0.018	0.0164
14	0.053	0.052
21	0.057	0.0556
28	0.0985	0.0953
42	0.1668	0.1553
56	0.2326	0.1901
70	0.2965	0.2361
84	0.3686	0.2446
112	0.3849	0.259
140	0.4403	0.2803
168	0.4511	0.2868
196	0.5157	0.895

References

Cameron TP, Hickman RL, Kornreich MR, Tarone RE (1985). History, survival, and growth patterns of B6C3F1 mice and F344 rats in the National Cancer Institute Carcinogenesis Testing Program. *Fundam Appl Toxicol* **5**(3):526-38.

Poiley SM (1972). Growth tables for 66 strains and stocks of laboratory animals. *Lab Anim Sci* **22**(5):758-79.

U.S. Environmental Protection Agency (U.S. EPA) (1988). *Recommendations for and Documentation of Biological Values in Risk Assessment*. Cincinnati, Ohio: U.S. EPA, EPA/600/6-87-008.

Appendix B

Unadjusted ASFs for Multi-Window Studies

Age sensitivity factor (ASF) distribution statistics derived from multi-window study datasets obtained from cancer bioassay experiments in which separate groups of animals were exposed to chemical carcinogens during "early life" or "later life" age windows are presented here. For each multi-window dataset, the ASF distribution was computed as the quotient of the cancer potency distribution for those animals exposed during the early life period (e.g., prenatal, postnatal, or juvenile) and those exposed in later life (e.g., adult, or juvenile in cases where no adult exposure group was included).

Table B1 presents the unadjusted prenatal ASF distributions and study details for the multi-window datasets that included a prenatal exposure group, grouped by carcinogen. Table B2 presents the unadjusted postnatal ASF distributions and study details for the multi-window datasets that included a postnatal exposure group, grouped by carcinogen. Table B3 presents the unadjusted juvenile ASF distributions and study details for the multi-window datasets that included a juvenile exposure group as the "early life" exposure, grouped by carcinogen.

Table B1. Prenatal Exposure Window: Estimated Age Sensitivity Factors (Unadjusted) for Different Chemicals

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Benzidine	Vesselinovitch et al. (1979a)	Mouse*	B6C3F1	Female	No	2	9.12E-01	0.000%	1.36E-01	4.52E-01	7.70E-01	1.22E+00	2.17E+00
		Mouse*	B6C3F1	Male	No	2	4.64E+01	0.000%	2.57E+01	3.54E+01	4.42E+01	5.49E+01	7.46E+01
Butylnitrosourea	Zeller et al. (1978)	Rat*	Sprague Dawley	Male/ Female	Yes	2	5.82E-01	0.000%	2.18E-01	3.74E-01	5.30E-01	7.30E-01	1.12E+00
Diethylstilbesterol (DES)	Turusov et al. (1992)	Mouse	CBA	Female	No	2	4.07E-01	0.000%	1.38E-01	2.54E-01	3.59E-01	5.02E-01	8.25E-01
Diethylnitrosamine (DEN)	Mohr et al. (1975)	Hamster	Syrian Golden	Female	No	2	1.94E+00	0.000%	1.01E+00	1.41E+00	1.80E+00	2.32E+00	3.34E+00
	Mohr et al. (1995)	Hamster	Syrian Golden	Female	No	2	5.01E-01	0.000%	2.86E-01	3.87E-01	4.78E-01	5.89E-01	7.95E-01
Dimethylnitrosamine (DMN)	Althoff et al. (1977)	Hamster	Syrian Golden	Male/ Female	Yes	2	7.84E+00	4.028%	2.40E-01	4.38E-01	6.86E-01	1.20E+00	1.64E+01
Di-n-propyl-nitrosamine (DPN)	Althoff et al. (1977)	Hamster	Syrian Golden	Male/ Female	Yes	2	1.47E-01	0.000%	6.40E-02	1.00E-01	1.34E-01	1.79E-01	2.76E-01
	Althoff and Grandjean (1979)	Hamster	Syrian Golden	Female	No	2	1.18E-01	0.000%	4.03E-02	7.55E-02	1.07E-01	1.49E-01	2.33E-01
1-Ethylnitrosobiuret	Druckrey and Landschutz (1971)	Rat	BD IX	Male/ Female	Yes	2	1.64E+01	0.000%	8.70E+00	1.19E+01	1.51E+01	1.94E+01	2.88E+01
					Yes	2	4.87E+00	0.000%	2.88E+00	3.78E+00	4.62E+00	5.68E+00	7.75E+00

Table B1. Continued. Prenatal Exposure Window

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Ethylnitrosourea (ENU)	Naito et al. (1981)	Rat*	Wistar	Female	No	2	3.28E+01	4.051%	6.55E+00	1.24E+01	2.03E+01	3.56E+01	2.66E+02
				Male	No	2	7.50E+00	0.000%	3.18E+00	4.84E+00	6.62E+00	9.14E+00	1.48E+01
	Tomatis et al. (1977)	Rat	BDVI	Female	No	2	2.89E+00	0.000%	1.20E+00	1.85E+00	2.54E+00	3.53E+00	5.76E+00
2-Hydroxypropyl-nitrosamine	Althoff and Grandjean (1979)	Hamster	Syrian Golden	Male/ Female	No	2	1.55E-01	0.000%	2.95E-02	8.34E-02	1.33E-01	2.00E-01	3.54E-01
3-Methyl-cholanthrene (3-MC)	Tomatis et al. (1971)	Mouse	CF-1	Female	Yes	2	6.49E-01	0.000%	4.20E-01	5.30E-01	6.26E-01	7.42E-01	9.53E-01
	Turusov et al. (1973)	Mouse	CF-1	Female	No	2	4.17E+00	0.000%	2.03E+00	2.92E+00	3.80E+00	5.01E+00	7.54E+00
4-(Methylnitros-amino)-1-(3-pyridyl)-1-butanone (NNK)	Anderson et al. (1989)	Mouse	C3H & B6C3F1[a]	Male/ Female[b]	Yes	2	1.66E-01	0.000%	6.18E-02	1.12E-01	1.56E-01	2.09E-01	3.06E-01
Safrole	Vesselinovitch et al. (1979a)	Mouse*	B6C3F1	Male	No	2	5.56E+01	1.485%	4.86E+00	1.92E+01	3.51E+01	6.32E+01	1.91E+02
	Vesselinovitch et al. (1979b)	Mouse*	B6C3F1	Female	Yes	2	3.37E+00	0.000%	1.12E+00	2.07E+00	3.03E+01	4.31E+00	6.81E+00
Urethane	Choudari Kommineni et al. (1970)	Rat*	MRC	Male/ Female	No	2	4.98E+00	1.031%	4.89E-01	1.80E+00	3.31E+00	5.91E+00	1.55E+01
Vinyl chloride	Maltoni et al. (1981)	Rat	Sprague Dawley	Male/ Female	Yes	2	2.57E+00	0.000%	1.28E+00	1.92E+00	2.46E+00	3.10E+00	4.19E+00

* Later life exposure group was dosed during the later part of the juvenile period.
[a] Pregnant C3H females were mated with C57BL males to produce B6C3F1 offspring.
[b] C3H adult females; B6C3F1 prenatal males.

Table B2. Postnatal Exposure Window: Estimated Age Sensitivity Factors (Unadjusted) for Different Chemicals

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Benzidine	Vesselinovitch et al. (1975b)	Mouse*	B6C3F1	Male	No	2	9.98E+01	0.000%	6.75E+01	8.23E+01	9.54E+01	1.12E+02	1.46E+02
	Vesselinovitch et al. (1979)	Mouse*	B6C3F1	Female	No	2	8.76E-01	0.000%	1.66E-01	4.34E-01	7.39E-01	1.17E+00	2.07E+00
				Male	No	2	1.95E+02	0.000%	1.21E+02	1.56E+02	1.88E+02	2.26E+02	2.98E+02
Benzo[a]pyrene	Truhaut et al. (1966)	Mouse*	Swiss	Male/ Female	No	2	6.20E-01	0.000%	2.55E-01	3.88E-01	5.31E-01	7.43E-01	1.28E+00
	Vesselinovitch et al. (1975a)	Mouse*	B6C3F1	Female	Yes	2	2.28E+00	0.000%	1.50E+00	1.86E+00	2.18E+00	2.60E+00	3.42E+00
				Male	Yes	2 & 3[c]	1.96E+00	0.000%	1.42E+00	1.70E+00	1.93E+00	2.18E+00	2.61E+00
			C3AF1	Female	Yes	2	1.90E+00	0.000%	1.14E+00	1.50E+00	1.82E+00	2.21E+00	2.94E+00
				Male	Yes	2	2.06E+00	0.000%	1.20E+00	1.59E+00	1.94E+00	2.40E+00	3.30E+00
1,1-Bis(*p*-Chlorophenol)-2,2,2-trichloroethane (DDT)	Vesselinovitch et al. (1979a)	Mouse*	B6C3F1	Male	No	2	1.46E+01	0.000%	8.43E-01	5.61E+00	9.68E+00	1.56E+01	3.25E+01
Butylnitrosourea	Zeller et al. (1978)	Rat*	Sprague Dawley	Male/ Female	Yes	2	3.99E+00	0.000%	2.46E+00	3.17E+00	3.80E+00	4.60E+00	6.14E+00
Dibutylnitrosamine	Wood et al. (1970)	Mouse	IF x C57	Female	Yes	2	7.49E+01	0.000%	3.32E+01	4.96E+01	6.64E+01	9.04E+01	1.45E+02
				Male	Yes	2	8.04E+01	0.000%	3.53E+01	5.25E+01	7.08E+01	9.73E+01	1.59E+02

Table B2. Continued. Postnatal Exposure Window

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Diethylnitrosamine (DEN)	Rao and Vesselinovitch (1973)	Mouse*	B6C3F1	Male	No	2	2.8E+01	0.000%	1.25E+01	1.84E+01	2.47E+01	3.37E+01	5.45E+01
	Vesselinovitch et al. (1984)	Mouse*	B6C3F1	Female (day 1)[a]	Yes	2 & 3[c]	2.28E+00	0.000%	1.57E+00	1.92E+00	2.22E+00	2.57E+00	3.20E+00
				Male (day 1)[a]	Yes	2 & 3[c]	5.23E+00	0.000%	3.67E+00	4.46E+00	5.12E+00	5.88E+00	7.18E+00
				Female (day 15)[b]	Yes	2 & 3[c]	1.75E+00	0.000%	1.20E+00	1.47E+00	1.71E+00	1.98E+00	2.47E+00
				Male (day 15)[b]	Yes	2 & 3[c]	4.50E+00	0.000%	3.22E+00	3.87E+00	4.41E+00	5.03E+00	6.10E+00
			C3AF1	Female (day 1)[a]	Yes	2	1.27E+00	0.000%	6.60E-01	9.40E-01	1.20E+00	1.52E+00	2.15E+00
				Male (day 1)[a]	Yes	2	2.90E+00	0.000%	1.75E+00	2.30E+00	2.79E+00	3.37E+00	4.42E+00
				Female (day 15)[b]	Yes	2	6.00E-01	0.000%	3.10E-01	4.50E-01	5.70E-01	7.20E-01	1.01E+00
				Male (day 15)[b]	Yes	2	1.69+00	0.000%	1.01E+00	1.34E+00	1.62E+00	1.97E+00	2.59E+00
7,12-Dimethyl-benz[a]anthracene (DMBA)	Meranze et al. (1969)	Rat	Fels-Wistar	Female	Yes	2	2.24E+01	0.248%	6.89E+00	1.03E+01	1.44E+01	2.12E+01	4.68E+01
				Male	Yes	2	1.59E+01	0.000%	6.03E+00	9.61E+00	1.35E+01	1.93E+01	3.37E+01
	Walters (1966)	Mouse	BALB/c	Female	No	2	1.30E+00	0.000%	6.78E-01	9.59E-01	1.22E+00	1.55E+00	2.20E+00
				Male	No	2	6.96E-01	0.000%	3.21E-01	4.81E-01	6.39E-01	8.46E-01	1.27E+00
1,2-Dimethylhydrazine	Martin et al. (1974)	Rat*	BDIX	Male/ Female	No	2	2.47E-01	0.000%	6.33E-02	1.31E-01	2.05E-01	3.15E-01	5.71E-01

Table B2. Continued. Postnatal Exposure Window

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
1-Ethylnitrosobiuret	Druckrey and Landschutz (1971)	Rat	BD IX	Male/ Female	Yes	2	1.34E+01	0.000%	6.13E+00	9.31E+00	1.24E+01	1.63E+01	2.42E+01
Ethylnitrosourea (ENU)	Naito et al. (1985)	Gerbil*	Mongolian	Female	No	2	7.64E-01	0.000%	1.53E-01	3.60E-01	5.94E-01	9.66E-01	1.91E+00
				Male	No	2	4.37E+00	2.975%	8.20E-01	1.61E+00	2.70E+00	4.78E+00	1.97E+01
	Bosch (1977)	Rat*	WAG	Female	Yes	2	5.03E+00	0.000%	1.80E+00	2.98E+00	4.28E+00	6.20E+00	1.08E+01
				Male	Yes	2	3.51E+00	0.000%	1.07E+00	1.85E+00	2.78E+00	4.29E+00	8.40E+00
	Naito et al. (1981)	Rat*	Wistar	Female	Yes	2	2.28E+01	4.051%	5.30E+00	9.24E+00	1.46E+01	2.46E+01	1.87E+02
				Male	Yes	2	2.82E+00	0.000%	1.35E+00	1.94E+00	2.55E+00	3.40E+00	5.20E+00
	Vesselinovitch et al. (1974)	Mouse*	B6C3F1	Female (day 1)[a]	Yes	2	1.98E+00	0.000%	1.32E+00	1.64E+00	1.91E+00	2.25E+00	2.88E+00
				Male (day 1) [a]	Yes	2	1.80E+00	0.000%	1.35E+00	1.59E+00	1.77E+00	1.98E+00	2.33E+00
				Female (day 15)[b]	Yes	2	1.22E+00	0.000%	9.09E-01	1.07E+00	1.20E+00	1.35E+00	1.59E+00
				Male (day 15) [b]	Yes	2	2.65E+00	0.000%	1.89E+00	2.27E+00	2.59E+00	2.96E+00	3.64E+00
			C3AF1	Female	Yes	2	2.94E+00	0.000%	1.93E+00	2.39E+00	2.81E+00	3.33E+00	4.41E+00
				Male (day 1) [a]	Yes	2	6.95E+00	0.000%	4.32E+00	5.55E+00	6.65E+00	8.01E+00	1.06E+01
				Male (day 15)[b]	Yes	2	4.90E+00	0.000%	3.19E+00	4.01E+00	4.72E+00	5.59E+00	7.23E+00
3-Hydroxyxanthine	Anderson et al. (1978)	Rat	Wistar	Female	No	2	8.15E+00	1.551%	0.00E+00	2.19E+00	4.60E+00	8.77E+00	2.95E+01
3-Methyl-cholanthrene (3-MC)	Klein (1959)	Mouse	A/He	Female	Yes	2	4.58E+00	0.000%	2.14E+00	3.14E+00	4.15E+00	5.51E+00	8.50E+00
				Male	Yes	2	5.48E+00	0.000%	2.95E+00	4.06E+00	5.12E+00	6.50E+00	9.26E+00

Table B2. Continued. Postnatal Exposure Window

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Methylnitrosourea (MNU)	Terracini and Testa (1970)	Mouse*	B6C3F1	Female	Yes	2	1.29E+00	0.000%	7.87E-01	1.03E+00	1.24E+00	1.49E+00	1.96E+00
				Male	Yes	2	3.36E+00	0.000%	2.07E+00	2.69E+00	3.23E+00	3.88E+00	5.07E+00
	Terracini et al. (1976)	Mouse	C3Hf/Dp	Female	Yes	2	1.07E+00	0.000%	5.90E-01	8.28E-01	1.03E+00	1.26E+00	1.69E+00
				Male	Yes	2	8.21E-01	0.000%	5.48E-01	6.83E-01	7.96E-01	9.32E-01	1.18E+00
β-Propiolactone	Chernozemski and Warwick (1970)	Mouse	B6AF1	Female	No	2	1.29E+00	0.525%	3.38E-01	6.38E-01	9.77E-01	1.52E+00	3.13E+00
				Male	No	2	1.07E+01	0.983%	2.39E+00	4.01E+00	5.97E+00	9.27E+00	2.14E+01
Safrole	Vesselinovitch et al. (1979a)	Mouse*	B6C3F1	Male	No	2	1.29E+02	1.485%	3.69E+01	5.94E+01	8.74E+01	1.39E+02	3.94E+02
	Vesselinovitch et al. (1979b)	Mouse*	B6C3F1	Male	No	2	3.56E+02	8.154%	3.51E+01	6.18E+01	1.02E+02	2.14E+02	Indeterminate
Tetrachlorodibenzodioxin (TCDD)	Della Porta et al. (1987)	Mouse*	B6C3F1	Female	Yes	2	1.88E+00	0.000%	1.36E-01	6.94E-01	1.46E+00	2.58E+00	5.19E+00
				Male	Yes	2	2.41E-01	0.000%	6.44E-02	1.53E-01	2.26E-01	3.11E-01	4.65E-01
Urethane	Choudari Kommineni et al. (1970)	Rat*	MRC	Male/ Female	Yes	2	1.39E+01	1.031%	4.95E+00	7.40E+00	1.02E+01	1.51E+01	3.56E+01
Vinyl chloride	Maltoni et al. (1981)	Rat	Sprague Dawley	Male/ Female	Yes	2	6.18E+00	0.000%	4.58E+00	5.41E+00	6.08E+00	6.85E+00	8.13E+00

* Later life exposure group was dosed during the later part of the juvenile period.
[a] Animals in the postnatal exposure group were dosed on day 1 of life.
[b] Animals in the postnatal exposure group were dosed on day 15 of life.
[c] Number of model parameters differed by tumor site.

Table B3. Juvenile Exposure Window: Estimated Age Sensitivity Factors (Unadjusted) for Different Chemicals

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
7,12-Dimethyl-benz[a]anthracene (DMBA)	Meranze et al. (1969)	Rat	Fels-Wistar	Female	Yes	2	9.74E+00	0.248%	2.79E+00	4.37E+00	6.17E+00	9.24E+00	2.07E+01
				Male	No	2	1.24E+00	0.000%	3.21E-01	6.31E-01	9.95E-01	1.55E+00	2.96E+00
Dimethylnitrosamine (DMN)	Noronha and Goodall (1984)	Rat	CRL/CDF	Male	Yes	2	1.80E+00	0.000%	1.14E+00	1.46E+00	1.73E+00	2.07E+00	2.70E+00
3-Hydroxyxanthine	Anderson et al. (1978)	Rat	Wistar	Female	No	2	1.55E+00	1.551%	9.89E-02	4.81E-01	9.03E-01	1.63E+00	5.28E+00
Methylnitrosourea (MNU)	Grubbs et al. (1983)	Rat	Sprague Dawley	Female[a+]	Yes	2	3.57E+00	0.000%	2.25E+00	2.88E+00	3.43E+00	4.11E+00	5.39E+00
				Female[b]	Yes	2	1.11E+01	0.000%	6.61E+00	8.64E+00	1.05E+01	1.29E+01	1.77E+01
Urethane	Choudari Kommineni et al. (1970)	Rat*	MRC	Male/ Female	No	2	7.86E-01	1.031%	2.86E-02	2.92E-01	5.42E-01	9.41E-01	2.39E+00

***** Later life exposure group was dosed during the later part of the juvenile period.
[+] MNU dataset selected for generation of juvenile ASF mixture frequency distribution; see text for explanation.
[a] Animals in the adult exposure group were dosed from day 80 to 87.
[b] Animals in the adult exposure group were dosed from 140 to 147.

Appendix C

ASF Mixture Frequency Distributions from Methods 1—3 for the Multi-Window Studies

This appendix presents the detailed findings for the ASF mixture frequency distributions generated using Methods 1, 2 and 3 for the prenatal, postnatal, and juvenile exposure windows from the multi-window studies. As described in the Methods section, in order to derive the ASF mixture distribution for each early-life exposure window, each chemical in the data set was equally likely to be sampled, and each chemical was represented by a single ASF distribution. When there were multiple ASFs (representing multiple studies) on a chemical, three different methods were used to sample from them to derive the ASF mixture distribution for the chemical. Using Method 1, each of the ASF distributions available for a chemical is equally likely to be sampled. Using Method 2, each of the ASF distributions available for a chemical is sampled based upon an inverse-variance weighting scheme, where the variance is calculated for the distribution of the logarithm of the ASF, Var[logASF], and the likelihood that an ASF is sampled is proportional to 1/Var(log[ASF]). Using Method 3, the ASF distribution with the largest median is used as the representative "mixture" ASF distribution to represent the chemical.

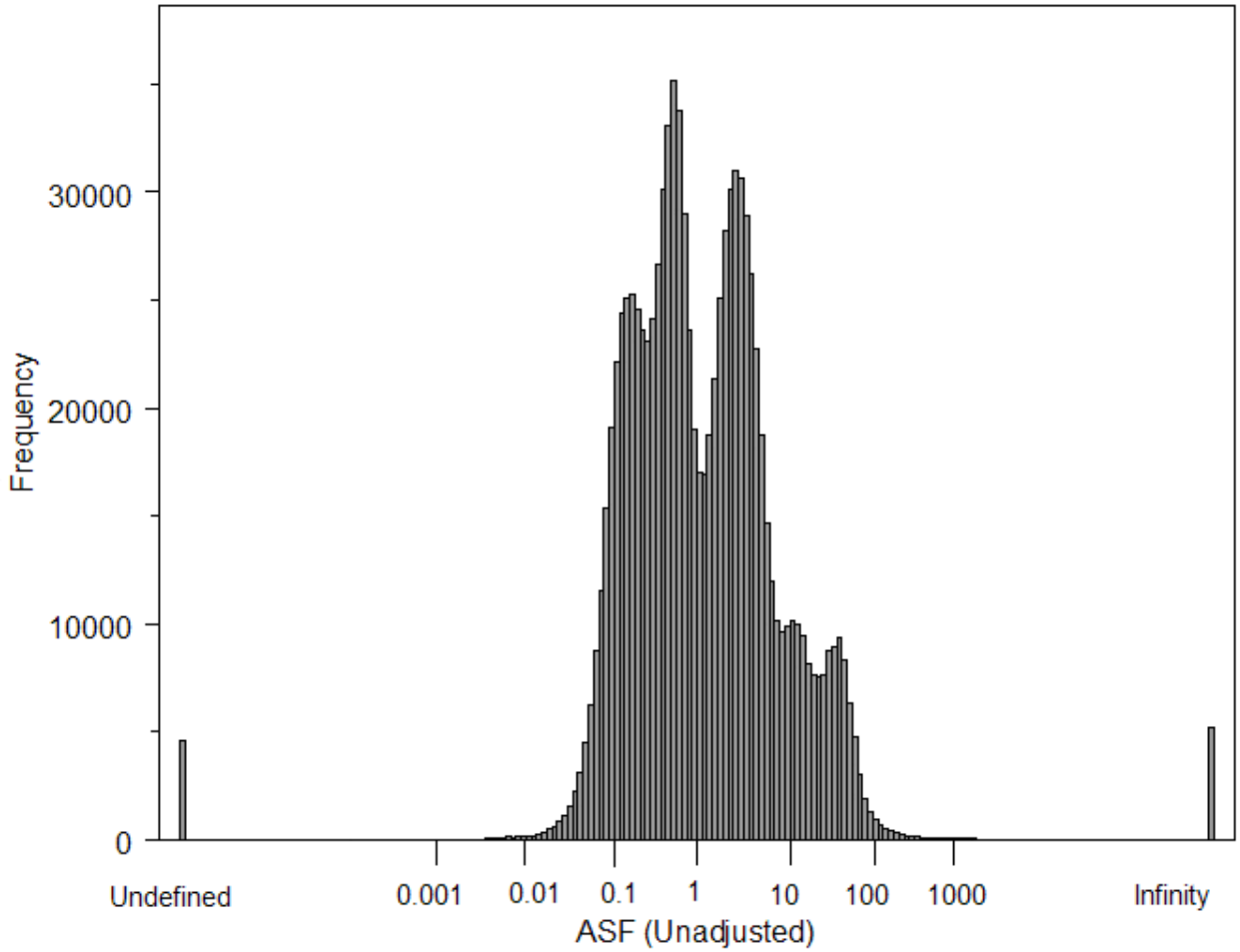
Prenatal ASF Mixture Distributions

Method 1: Chemicals Equally Weighted and Within Each Chemical Equal Weight per Study.

Figure C-1a shows the prenatal ASF mixture frequency distribution generated using Method 1. The frequency distribution is multi-modal (four modes), at 0.15, 0.54, 3.65, and 47.86. The largest peak of the frequency distribution is an ASF value of 0.54. The smallest mode, at an ASF value of 0.15, is primarily composed of ASF values from the following chemicals: di-*n*-propylnitrosamine, 2-hydroxypropylnitrosamine, and NNK. These chemicals display confidence intervals that indicate the true value of the ASF is statistically significantly less than 1.0 (at the 0.05 level; see also Fig. 1). The second mode, with a value of 0.54, is comprised primarily of ASF values from chemicals whereby a bulk of their ASF distributions lie below 1.0, yet the 90% upper confidence bound may be slightly greater than 1.0. These chemicals are as follows:

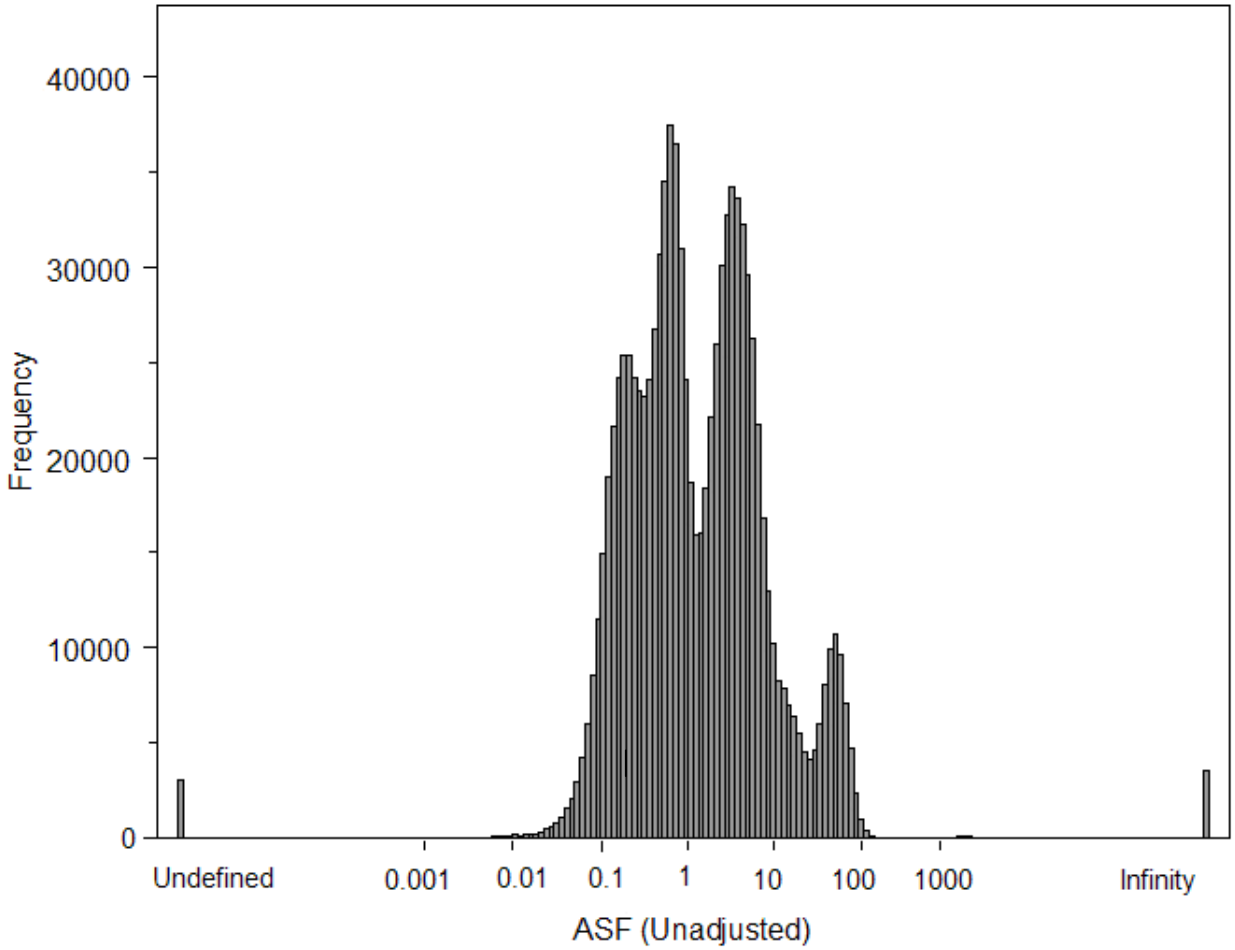
benzidine (female mouse), butylnitrosourea, DES, DEN (one of the two female hamster studies), dimethylnitrosamine, and 3-MC (one of the two female mouse studies). The third mode, with a value of 3.65, consists primarily of ASF values from chemicals whereby a bulk of their ASF distributions lie above 1.0 yet their upper 90% confidence bound is generally not greater than 10. These chemicals are as follows: DEN (one of the two female hamster studies), ENU (one of two female rat studies), 3-MC (one of the two female mouse studies), safrole (female mouse), urethane, and vinyl chloride. The largest mode is primarily composed of ASF values from the following chemicals: benzidine (male mouse), 1-ethylnitrosobiuret, ENU (male rat, one of two female rat studies), and safrole (male mouse). These chemicals display confidence intervals that indicate the true value of the ASF is statistically significantly greater than 1.0 (at the $p \leq 0.05$ level).

Figure C-1a. Method 1 Prenatal ASF Mixture Frequency Distribution – Equally Weighted Chemicals, Equally Weighted Studies



Method 2: Chemicals Equally Weighted and Within Each Chemical Inverse-Variance Weighting of Studies. Figure C-1b shows the prenatal ASF mixture frequency distribution generated using Method 2. The prenatal ASF mixture frequency distribution is multi-modal (four modes). The modes of the frequency distribution are 0.14, 0.52, 3.63, and 47.86. The largest peak of the frequency distribution is an ASF value of 0.52. The general shape of this prenatal ASF mixture frequency distribution is similar to that generated using Method 1 (i.e., all chemicals equally represented in the mixture distribution). Of those chemicals that had more than a single ASF dataset representing them, unless there were appreciable fold-differences across the studies, datasets within a chemical were generally sampled from equally using Method 2. In instances where there were fold differences across datasets within a chemical, the Method assigns datasets with the greatest variability (log space) the smallest weights in comparison to other datasets with less variability (log space). Chemicals that have multiple prenatal studies representing them that have fold-differences such that they are not equally sampled are benzidine, ENU, and safrole. The greatest departure between the ASF mixture frequency distributions generated using Method 1 and Method 2 is attributed to the datasets associated with these chemicals.

Figure C-1b. Method 2 Prenatal ASF Mixture Frequency Distribution – Equally Weighted Chemicals, Inverse-Variance Weighting of Studies



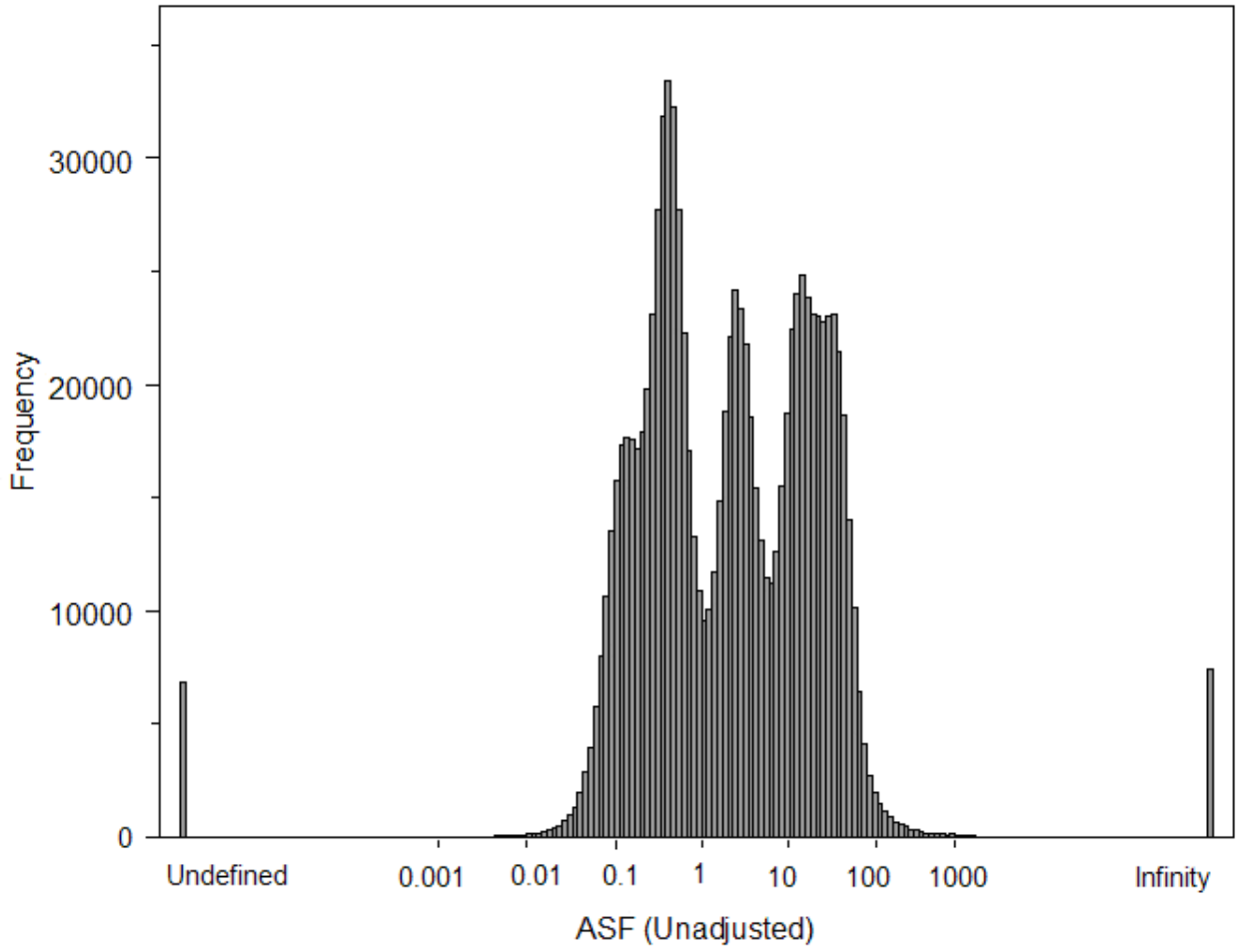
Method 3: Chemicals Equally Weighted, Single Study Represents Each Chemical.

Figure C-1c shows the prenatal ASF mixture frequency distribution generated using Method 3. The dataset selected as representative of each chemical was the one with the largest median in the ASF distribution. The prenatal ASF mixture frequency distribution is multi-modal (five modes). This distribution looks somewhat different than those shown in Figures C-1a and C-1b; it is more disperse and the modes of the distribution are more peaked for larger ASF values. The modes of this prenatal ASF mixture frequency distribution are 0.15, 0.53, 3.60, 19.12 and 47.98. The largest peak of this distribution is the ASF value of 0.53.

Of those chemicals that had more than a single study representing them, the study with the largest median tended to also have the largest variance. As a result, the mixture frequency distribution resulting from Method 3 tends to be more spread out and shifted toward the right.

The chemicals contributing to the peak with value 0.15 are di-n-propylnitrosamine, 2-hydroxypropylnitrosamine, and NNK. The chemicals primarily contributing to the mode with value 0.53 are butylnitrosourea and DES. The next largest peak with a value of 3.60 is comprised of the chemicals DEN, dimethylnitrosamine, 3-MC, urethane and vinyl chloride. The peaks with the largest modes (values of 19.12 and 47.98) consist of the chemicals benzidine, 1-ethylnitrosobiuret, ENU, and safrole. All of the studies that comprise the two peaks with the largest modes display confidence intervals that indicate the true value of the ASF is statistically significantly greater than 1 (at the 0.05 level).

Figure C-1c. Method 3 Prenatal ASF Mixture Frequency Distribution - Equally Weighted Chemicals, Single Study Represents Each Chemical

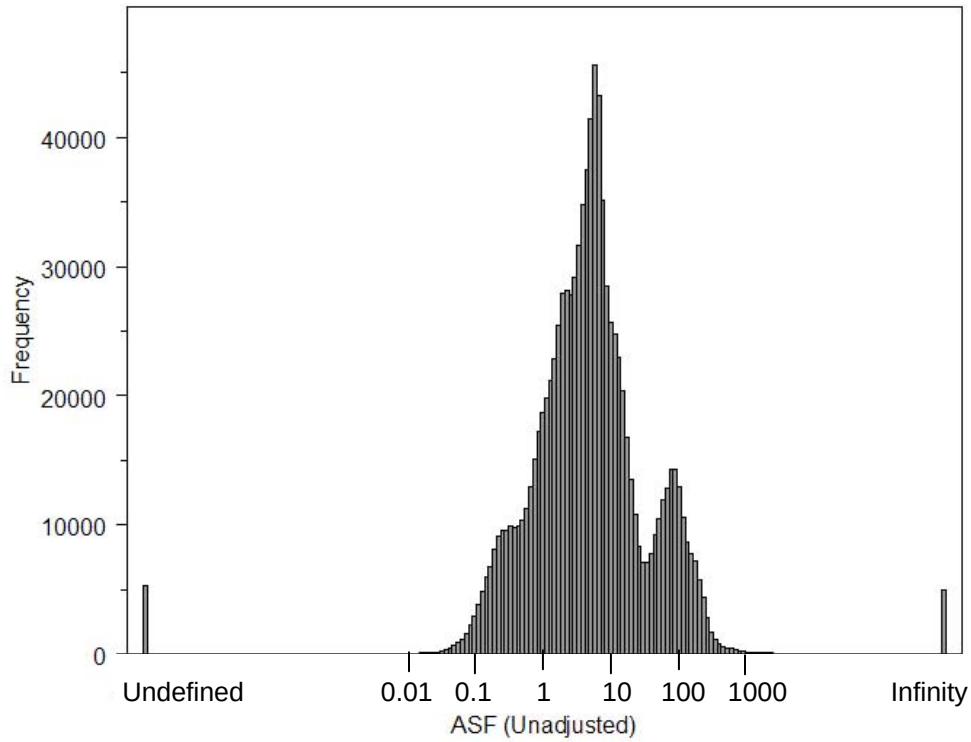


Postnatal ASF Mixture Distributions

Method 1: Chemicals Equally Weighted and Within Each Chemical Equal Weight per Study.

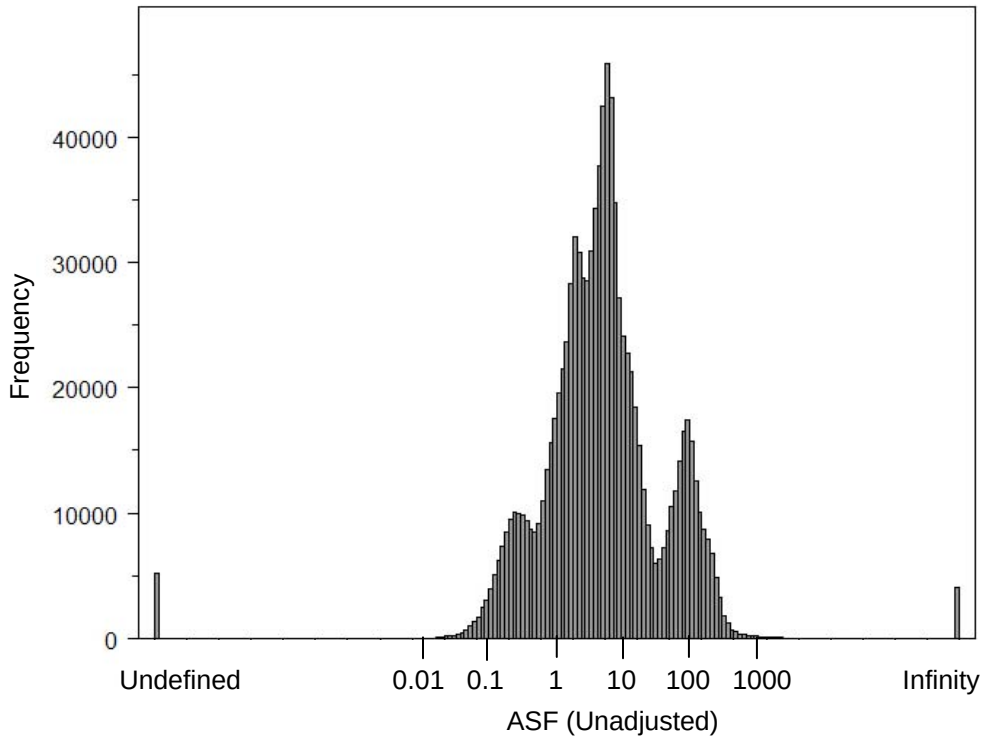
Figure C-2a shows the postnatal ASF mixture frequency distribution generated using Method 1. The ASF frequency distribution has three modes, at 0.61, 8.66, and 96.49, with the largest peak at 8.66. The smallest mode, with a value of 0.61, is primarily composed of ASF values from the two studies with the 95% upper bound below the ASF value of 1.0. The second mode, with a value of 8.66, is comprised primarily of ASF values from chemicals with the bulk of their ASF distributions above one, but 95% upper confidence bounds less than 10: benzo[a]pyrene, butylnitrosourea, DEN, ENU, 3-MC, and MNU. The ASFs for studies on these chemicals contribute the majority of the mass at the center of the distribution. The third mode, with a value of 96.49, consists primarily of chemicals with ASF values centered around 100: benzidine (one male mouse study), dibutylnitrosamine, and safrole. The ASFs for these cases are statistically significantly greater than 10 (at the $p = 0.05$ level).

Figure C-2a. Method 1 Postnatal ASF Mixture Frequency Distribution – Equally Weighted Chemicals, Equally Weighted Studies



Method 2: Chemicals Equally Weighted and Within Each Chemical Inverse-Variance Weighting of Studies. Figure C-2b shows the postnatal ASF mixture frequency distribution generated using Method 2. The postnatal ASF mixture frequency distribution has four modes, at 0.49, 1.43, 8.66, and 95.55. As with Method 1, the largest has an ASF value of 8.66, and its general shape is similar to the one generated using Method 1 (Figure C-2a). The main difference is that the Method 2 distribution is slightly more spread out with more defined peaks, and the peaks tend to be more elevated. The higher peaks are due to the studies within a chemical that have smaller fold differences being weighted more heavily than those studies with greater variability (e.g. benzidene, benzo[a]pyrene, DEN, and ENU). However, the studies with greater variability (log space) are still contributing to the frequency distribution. The studies with the most variability (log space) and the largest ASF values contribute to the enhanced variability of Method 2 as compared to Method 1.

Figure C-2b. Method 2 Postnatal ASF Mixture Frequency Distribution – Equally Weighted Chemicals, Inverse-Variance Weighting of Studies



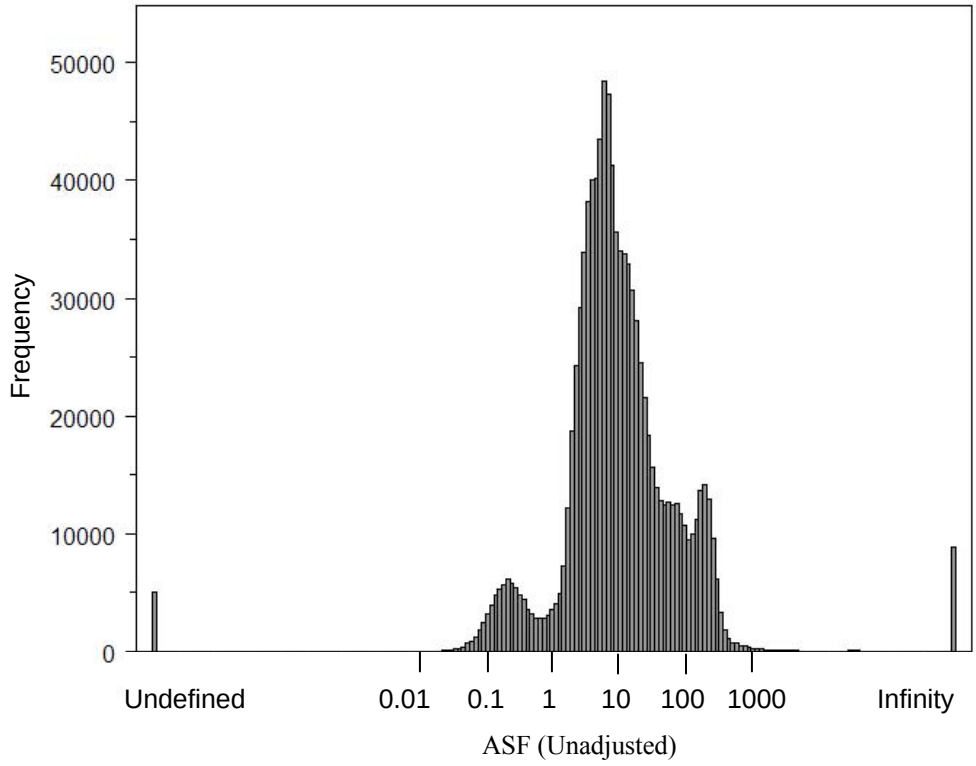
Method 3: Chemicals Equally Weighted, Single Study Represents Each Chemical.

Figure C-2c shows the postnatal ASF mixture frequency distribution generated using Method 3. The dataset selected as representative of each chemical was the one with the largest median in the ASF distribution. The postnatal ASF mixture frequency distribution again has four modes,

0.58, 8.96, 97.83, and 163.79. It has two very distinct peaks and is more skewed to the right than those shown in Figures C-2a and C-2b. The largest peak of this frequency distribution is an ASF value of 8.96.

For chemicals where there is significant study-to-study variability, the effect of selecting the distribution with the largest median exaggerates the percentiles of the resultant mixture frequency distribution. This effect is most pronounced for the chemicals benzidine, DEN, DMBA, ENU, and β-propiolactone.

Figure C-2c. Method 3 Postnatal ASF Mixture Frequency Distribution - Equally Weighted Chemicals, Single Study Represents Each Chemical



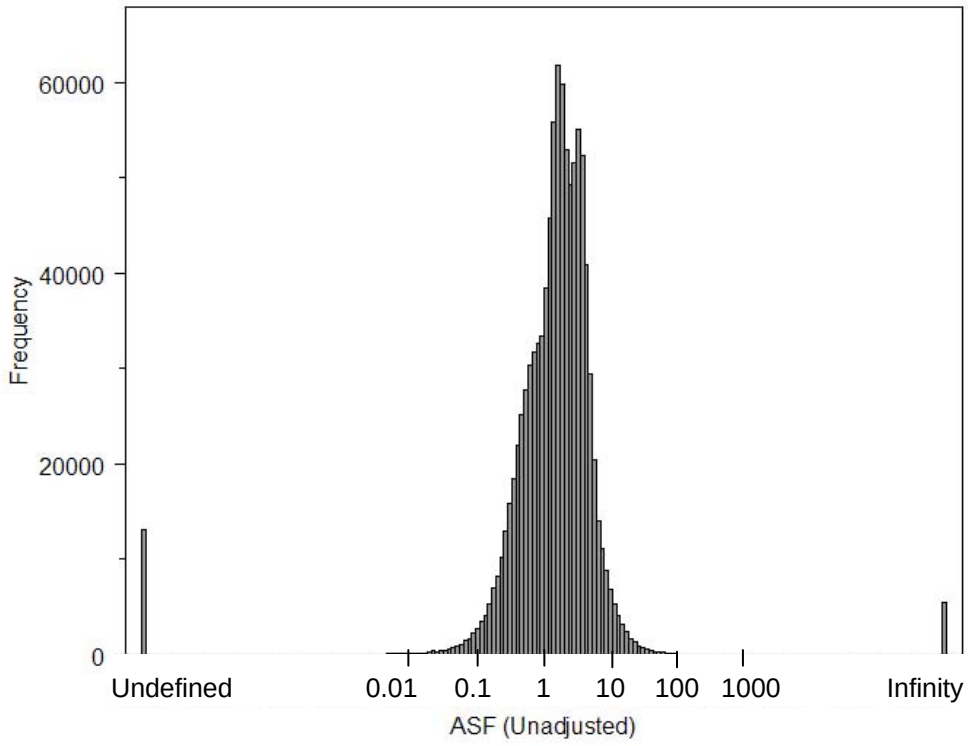
Juvenile ASF Mixture Distributions

Method 1: Chemicals Equally Weighted and Within Each Chemical Equal Weight per Study.

Figure C-3a shows the juvenile ASF mixture frequency distribution generated using Method 1. The frequency distribution is bi-modal, with modes at 1.58 and 2.05. The largest peak of the distribution is an ASF value of 1.58. By sorting the chemicals from smallest to largest based upon the value of the lower confidence bound, we can approximately determine each chemical's

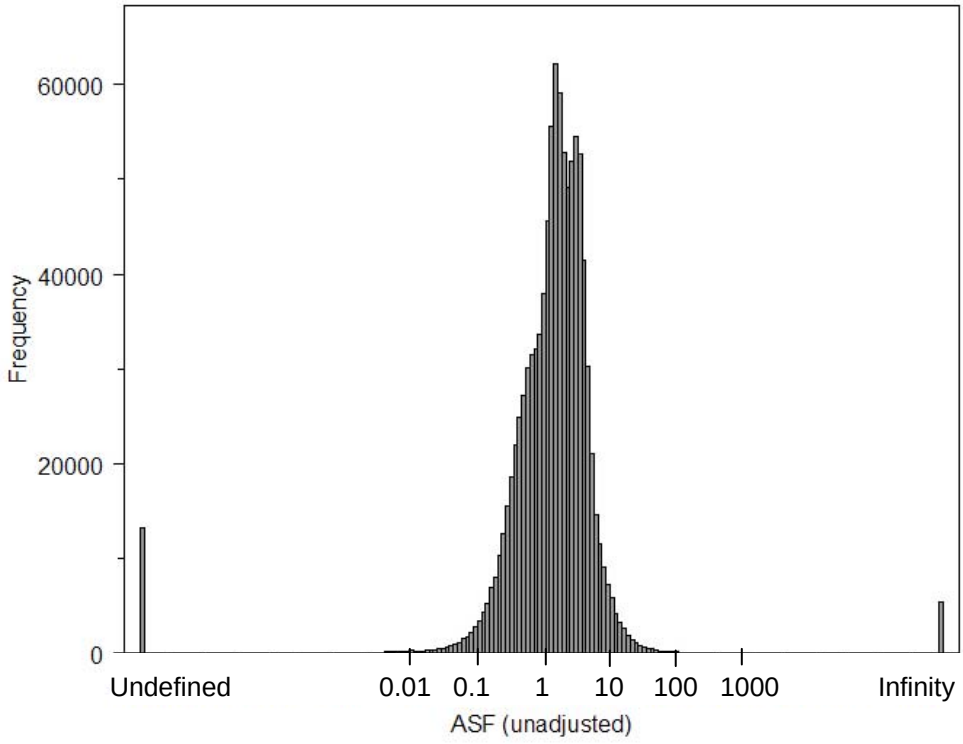
contribution to the percentiles of the ASF mixture frequency distribution. Urethane and 3-hydroxyxanthine are the largest contributors to the lower percentiles of the mixture frequency distribution. Conversely, MNU and the DMBA female rat datasets are the largest contributors to the highest percentiles of the mixture frequency distribution. The male rat DMBA dataset and the DMN dataset (also in male rats) comprise the middle area of the distribution.

Figure C-3a. Method 1 Juvenile ASF Mixture Frequency Distribution – Equally Weighted Chemicals, Equally Weighted Studies



Method 2: Chemicals Equally Weighted and Within Each Chemical Inverse-Variance Weighting of Studies. Figure C-3b shows the juvenile ASF mixture frequency distribution generated using Method 2. The frequency distribution is bi-modal, with modes at 1.57 and 2.08. The largest peak of the distribution is an ASF value of 1.57. This ASF distribution is practically identical to the ASF distribution derived via Method 1.

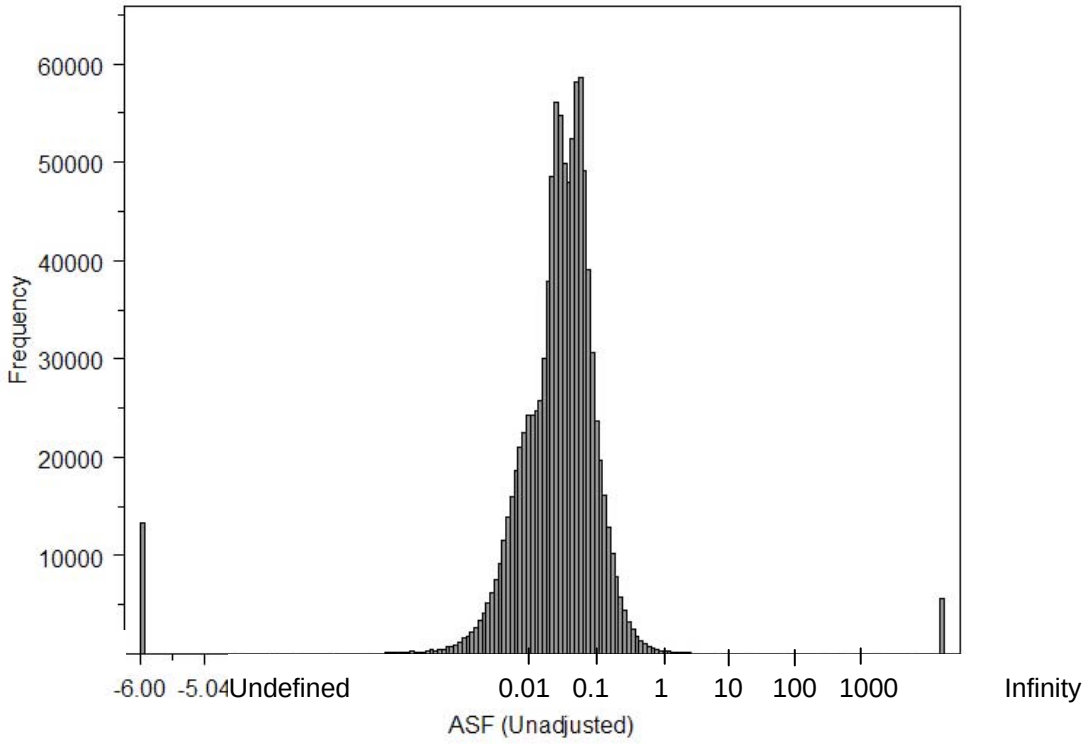
Figure C-3b. Method 2 Juvenile ASF Mixture Frequency Distribution – Equally Weighted Chemicals, Inverse-Variance Weighting of Studies



Method 3: Chemicals Equally Weighted, Single Study Represents Each Chemical.

Figure C-3c shows the juvenile ASF mixture frequency distribution generated using Method 3. The juvenile ASF mixture frequency distribution is bi-modal, and looks similar to that generated by Methods 1 and 2. However, the modes of this distribution, 1.59 and 2.37, are less peaked and are of similar height. The largest peak of this mixture frequency distribution is the ASF value of 2.37.

Figure C-3c. Method 3 Juvenile ASF Mixture Frequency Distribution - Equally Weighted Chemicals, Single Study Represents Each Chemical



Appendix D

DEN Case Study: Cancer Potency Distributions for DEN Experiments

DEN (N-nitrosodiethylamine) cancer potency distribution statistics derived from cancer bioassay experiments conducted in mice exposed to DEN during either the prenatal, postnatal, or juvenile age window are presented here. Table D1 presents the cancer potency distributions and study details for the prenatal exposure datasets. Table D2 presents the cancer potency distributions and study details for the postnatal exposure datasets. Table D3 presents the cancer potency distributions and study details for the juvenile exposure datasets.

Table D1. DEN Prenatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Anderson et al. (1989)	C3H/HeN	Female (540)[a]	0.0793739	0.0147744	0.0558226	0.0690785	0.0788648	0.0891974	0.10467
		Female (650)[a]	0.00135364	0.00149944	0	0.000151185	0.00091793	0.00200131	0.00440449
		Male (461)[a]	0.138321	0.0511987	0.0596149	0.101144	0.134968	0.171184	0.229449
		Male (644)[a]	0.00411408	0.00501051	0	0.000575143	0.00236785	0.0054626	0.0151794
Mohr and Althoff. (1965)	NMRI	Female	0.239892	0.067558	0.132634	0.192885	0.236059	0.283579	0.359286
		Male	0.187186	0.0701371	0.0756144	0.137731	0.18516	0.233584	0.306676
Vesselinovitch (1983)	B6C3F1	Female	0.00667806	0.00582567	0	0.00240222	0.00513474	0.00941687	0.0187249
		Male	0.00952546	0.00812867	0	0.00313266	0.00792549	0.0140185	0.0251028

[a] Day of sacrifice.

Table D2. DEN Postnatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Boberg et al. (1983)	B6C3F1	Male	48.3648	14.391	28.9842	38.0081	46.0943	56.3092	75.2761
Drinkwater and Ginsler (1986)	B6C3F1	Male	17.9418	4.73622	11.1826	14.5018	17.3425	20.7377	26.8706
	C3H/HeJ	Male	22.9791	7.17883	13.3745	17.8596	21.7639	26.8271	36.8345
			2.68143	0.555517	1.82759	2.28691	2.63839	3.03852	3.65828
Lai et al. (1985)	B6C3F2	Male	12.8913	2.39873	9.27649	11.1601	12.6851	14.41	17.2813
Rao and Vesselinovitch (1973)	B6C3F1	Female	1.41599	0.285257	0.978519	1.21049	1.39454	1.59902	1.93235
		Male	2.30206	0.661882	1.43057	1.82508	2.18762	2.65531	3.542
Turusov et al. (1973)	CF-1	Female	0.575921	0.131105	0.37814	0.481996	0.565362	0.65859	0.810561
		Male	0.830932	0.174098	0.565934	0.707333	0.818634	0.941348	1.13883
Vesselinovitch et al. (1984)	B6C3F1	Female (Day 1)[a]	0.589447	0.062358	0.491627	0.545742	0.586343	0.63034	0.697702
		Male (Day 1) [a]	1.03983	0.148035	0.808426	0.93473	1.03146	1.13696	1.29788
		Female (Day 15)[b]	0.453917	0.051127	0.374289	0.417738	0.451081	0.48722	0.543127
		Male (Day 15) [b]	0.894762	0.115637	0.717843	0.81268	0.887008	0.968949	1.09932

Table D2. Continued. DEN Postnatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Vesselinovitch et al. (1984)	C3AF1	Female (Day 1) [a]	0.641045	0.111376	0.469409	0.562094	0.634021	0.712722	0.837305
		Male (Day 1) [a]	1.11429	0.173993	0.835839	0.993194	1.10931	1.22972	1.41043
		Female (Day 15) [b]	0.303322	0.050107	0.224424	0.267995	0.300956	0.336305	0.390135
		Male (Day 15) [b]	0.649307	0.106642	0.480839	0.574691	0.644526	0.719069	0.834195
Vesselinovitch (1980)	B6C3F1	Male	3.07401	0.452323	2.36812	2.75378	3.04908	3.3669	3.88832

[a] Mice were dosed on day 1 of life.
[b] Mice were dosed on day 15 of life.

Table D3. DEN Juvenile Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Rao and Vesselinovitch (1973)	B6C3F1	Male	0.093411	0.031799	0.048166	0.070136	0.089942	0.113182	0.15094
Vesselinovitch et al. (1984)	B6C3F1	Female	0.267868	0.049742	0.191397	0.232495	0.26428	0.299789	0.356424
		Male	0.203009	0.029221	0.157292	0.182313	0.201531	0.22207	0.254173
	C3AF1	Female	0.555707	0.178219	0.313719	0.427389	0.527989	0.654242	0.88766
		Male	0.40558	0.094585	0.268191	0.337805	0.395187	0.462985	0.579307

Appendix E

ENU Case Study: Cancer Potency Distributions for ENU Experiments

ENU (N-ethyl-N-nitrosourea) cancer potency distribution statistics derived from cancer bioassay experiments conducted in mice exposed to ENU during either the prenatal, postnatal, or juvenile age window are presented here. Table E1 presents the cancer potency distributions and study details for the prenatal exposure datasets. Table E2 presents the cancer potency distributions and study details for the postnatal exposure datasets. Table E3 presents the cancer potency distributions and study details for the juvenile exposure datasets.

Table E1. ENU Prenatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Study	Strain	Gender	Mean	SD	Percentiles				
					5th	25th	50th	75th	95th
Diwan et al. (1974)	AKR/J x SWR/J	Female	1.52277	0.531149	0.796624	1.14448	1.44501	1.81488	2.52135
		Male	0.788833	0.242592	0.408852	0.617535	0.777224	0.946775	1.21021
	SWR/J x AKR/J	Female	6.40048	1.26518	4.48233	5.50555	6.30745	7.19692	8.64127
		Male	8.23676	2.02853	5.41389	6.7886	7.96423	9.37737	12.0313
Kauffman (1976)	Swiss	Female (Day -7)[a]	2.75745	0.780679	1.70604	2.20068	2.63687	3.17936	4.26637
		Female (Day -6)[a]	2.73481	0.777314	1.68615	2.1712	2.60713	3.15855	4.24545
		Female (Day -5)[a]	2.39602	0.773729	1.38175	1.83596	2.26666	2.8147	3.8993
		Female (Day -4)[a]	2.79589	0.781762	1.74426	2.23081	2.66744	3.2193	4.3065
		Female (Day -3)[a]	2.53857	0.770214	1.50408	1.98352	2.41683	2.95218	3.99008
Vesselinovitch et al. (1977)	B6C3F1	Female (Day -10)[a]	0.042928	0.00468297	0.0354941	0.0396959	0.0427621	0.0460137	0.0509013
		Female (Day -8)[a]	0.0886033	0.00963707	0.0736767	0.0817998	0.0880656	0.0948531	0.105386
		Female (Day -6)[a]	0.136846	0.0191498	0.107902	0.123231	0.135315	0.148804	0.171023
		Female (Day -4)[a]	0.083219	0.0122441	0.0645201	0.0744767	0.0823669	0.0910178	0.104993
		Male (Day -10)[a]	0.0508204	0.00566404	0.041823	0.0468659	0.0506208	0.0545794	0.0604567
		Male (Day -8)[a]	0.127622	0.0154249	0.103632	0.116711	0.126869	0.137618	0.154515
		Male (Day -6)[a]	0.286018	0.0598357	0.204919	0.243175	0.277137	0.319002	0.398503
		Male (Day -4)[a]	0.165365	0.0228038	0.131436	0.149108	0.16331	0.179562	0.206382
	C3B6F1	Female (Day -10)[a]	0.0235324	0.00539875	0.0151785	0.0197164	0.0232224	0.0270231	0.0329388
		Female (Day -8)[a]	0.111417	0.0169991	0.0860396	0.0992914	0.109892	0.121913	0.141993
		Female (Day -6)[a]	0.121747	0.0240692	0.0860168	0.104546	0.119536	0.13667	0.165114
		Female (Day -4)[a]	0.0729087	0.00911406	0.0587817	0.0664352	0.0723775	0.078822	0.0889698
		Male (Day -10)[a]	0.0356864	0.00744729	0.0242335	0.0304194	0.0352127	0.0404608	0.0488031
		Male (Day -8)[a]	0.167691	0.0313038	0.122511	0.14528	0.164215	0.186164	0.225405
		Male (Day -6)[a]	0.241567	0.0548256	0.167721	0.202249	0.233152	0.271325	0.345658
		Male (Day -4)[a]	0.083293	0.00962997	0.068251	0.0765158	0.0828024	0.0895821	0.0999465
Vesselinovitch (1983)	B6C3F1	Female	0.0188286	0.00433908	0.0123229	0.0156683	0.0184721	0.0215981	0.0266903
		Male	0.0311922	0.00595895	0.0220985	0.0269647	0.0307819	0.0350559	0.041544
Wiggenhauser and Schmahl (1987)	NMRI	Male & Female (Day -8)[a]	0.191795	0.0154062	0.166844	0.181108	0.191371	0.202129	0.217871
		Male & Female (Day -7)[a]	0.181807	0.0165413	0.155072	0.170363	0.181426	0.192894	0.209776
		Male & Female (Day -6)[a]	0.153851	0.0149143	0.129937	0.143407	0.153387	0.16381	0.179375

[a] Day of dosing in gestation, where day of birth is designated as day 1.

Table E2. ENU Postnatal Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Study	Strain	Gender	Mean	SD	Percentiles				
					5th	25th	50th	75th	95th
Anderson et al. (1989)	C3H/HeNCr MTV	Female (405)[a]	0.401602	0.0714822	0.295997	0.350593	0.394317	0.445627	0.531848
		Female (451)[a]	0.190949	0.0332242	0.139564	0.16738	0.18888	0.212447	0.249345
		Male (342)[a]	0.705296	0.160744	0.46968	0.589517	0.689687	0.803716	0.997279
		Male (397)[a]	0.409096	0.0684954	0.300275	0.361199	0.406761	0.454284	0.526448
Drinkwater and Ginsler (1986)	C3H/HeJ	Male	1.87256	0.619931	1.04439	1.42364	1.7664	2.21011	3.03312
	C57BL/6J	Male	0.193632	0.0706384	0.0924212	0.141821	0.185241	0.236855	0.326457
Naito et al. (1982)	A/He	Female	0.488979	0.113488	0.321961	0.407412	0.47754	0.558629	0.696155
		Male	0.53121	0.106117	0.369563	0.455872	0.524035	0.598363	0.71904
Pereira et al. (1985)	CD1	Female	0.453666	0.0963253	0.303665	0.384955	0.448654	0.516608	0.622827
		Male	0.650342	0.167153	0.37248	0.522933	0.660456	0.772229	0.914794
Schmahl (1988)	NMRI	Female	0.0511349	0.00593372	0.04167	0.0469975	0.0509134	0.0550801	0.061308
		Female	0.0813521	0.010729	0.0648204	0.0737127	0.0806609	0.0882508	0.100297
		Male	0.0819858	0.010706	0.0654327	0.0744036	0.0812874	0.088867	0.100923
		Male	0.113765	0.016685	0.0891173	0.101707	0.11222	0.124072	0.143985
Searle and Jones (1976)	A	Male & Female	0.102345	0.0307605	0.0492138	0.0821283	0.103482	0.123574	0.151421
	C57BL	Male & Female	0.246532	0.0422712	0.180748	0.217317	0.244314	0.273666	0.32047
	DBAF	Male & Female	0.123967	0.0202948	0.090089	0.110854	0.12422	0.13749	0.156851
	IF	Male & Female	0.118889	0.0283388	0.0747125	0.0992445	0.11737	0.137096	0.168199
Vesselinovitch et al. (1974)	B6C3F1	Female (Day 1)[b]	0.0901191	0.0182086	0.0653432	0.0770816	0.0873993	0.100164	0.124208
		Female (Day 15)[c]	0.0555416	0.00590128	0.0463567	0.0513833	0.0552225	0.0593717	0.0658145
		Male (Day 1)[b]	0.0784357	0.0094572	0.0638592	0.0718073	0.0778351	0.0844925	0.0950637
		Male (Day 15)[c]	0.115803	0.0193859	0.0886015	0.102078	0.113324	0.126753	0.151878
	C3AF1	Female	0.0162293	0.00195763	0.0130923	0.0148687	0.0161727	0.0175495	0.0195461
		Male (Day 1)[b]	0.0478472	0.00952346	0.03385	0.0410128	0.0468717	0.0537055	0.0652326
		Male (Day 15)[c]	0.0337552	0.00545562	0.0254094	0.0298703	0.0333879	0.0372465	0.0434698
Vesselinovitch (1983)	B6C3F1	Female	0.0325201	0.00615214	0.0229783	0.0281148	0.0321294	0.0365301	0.0435769
		Male	0.0695924	0.0120803	0.0509478	0.0609427	0.0687729	0.0774048	0.0913846

[a] Day of sacrifice.
[b] Mice were dosed on day 1 of life.
[c] Mice were dosed on day 15 of life.

Table E3. ENU Juvenile Mouse Studies: Cancer Potency Estimates in Units (cumulative mg/kg-bw)$^{-1}$

Reference	Strain	Gender	Mean	SD	----------Percentiles----------				
					5th	25th	50th	75th	95th
Vesselinovitch et al. (1973)	B6C3F1	Female	0.00126335	0.000460445	0.000605155	0.000927673	0.00121074	0.00153788	0.00212734
		Male	0.00337468	0.000804246	0.00213171	0.00279749	0.00331756	0.00389027	0.00481319
Vesselinovitch et al. (1974)	B6C3F1	Female	0.0463117	0.00618634	0.0367588	0.0419302	0.045902	0.0503072	0.0572276
		Male	0.0441913	0.00498978	0.0363954	0.04067	0.0439454	0.0474287	0.0529056
	C3AF1	Female	0.00579571	0.00122561	0.00380638	0.00495406	0.00577705	0.00661704	0.00784915
		Male	0.00713611	0.00130036	0.00503552	0.00623303	0.00711547	0.00800999	0.0093149
Vesselinovitch (1983)	B6C3F1	Female	0.00451849	0.00192539	0.00183844	0.00309386	0.00425096	0.00566614	0.00819086
		Male	0.00886785	0.00285617	0.00458294	0.00681412	0.00858931	0.0106642	0.0139855

Appendix F

Early Life Across-Window Studies of Two Non-Genotoxic Carcinogens

Early in life studies in which exposure of a given exposure group crossed multiple age windows were excluded from the main analyses presented in this document, as across-window exposures preclude derivation of age-at-exposure sensitivity measures for specific early life age windows. Some studies with early life across-window exposures have been included in the analyses of Barton *et al.* (2005), and can provide information on early life vs. later life sensitivity. This appendix presents the unadjusted early life age sensitivity factor (ASF) distribution statistics derived from analyses of experiments conducted in mice with two non-genotoxic carcinogens: diphenylhydantoin (Chhabra *et al.*, 1993a) and polybrominated biphenyls (Chhabra *et al.*, 1993ab). In these studies separate groups of animals were exposed to either diphenylhydantoin or polybrominated biphenyls across multiple "early life" windows (i.e., prenatal, postnatal and juvenile) or during the adult age windows. For the early life exposure groups, exposures began prior to conception, and continued throughout the prenatal, postnatal, and post-weaning periods, up to the age of eight weeks.

Table F1 presents the unadjusted early life ASF distributions and study details for these early life across-window datasets.

Table F1. Across-Window Studies: Estimated Age Sensitivity Factors (Unadjusted) for Two Non-Genotoxic Chemicals

Chemical	Reference	Species	Strain	Gender	Multi-site	Model para-meters	Mean	Infinite values	5th percentile	25th percentile	50th percentile	75th percentile	95th percentile
Diphenylhydan-toin	Chhabra et al. (1993a)	Mouse	B6C3F1	Female	No	2	2.14E+01	0.000%	2.46E+00	1.25E+01	2.00E+01	2.87E+01	4.42E+01
Polybrominated biphenyls	Chhabra et al. (1993b)	Mouse	B6C3F1	Female	No	2	3.10E+00	0.000%	1.59E+00	2.36E+00	2.99E+00	3.72E+00	4.96E+00
				Male	No	2	3.90E+00	0.000%	1.93E+00	2.85E+00	3.68E+00	4.72E+00	6.62E+00